Technical Bulletin 92 February 1933

University of Minnesota
Agricultural Experiment Station

Fundamental Food Requirements for the Growth of the Rat

VII. An Experimental Study of Inheritance as a Factor Influencing Food Utilization in the Rat

H. P. Morris, L. S. Palmer, Cornelia Kennedy
Division of Biochemistry

UNIVERSITY FARM, ST. PAUL

FUNDAMENTAL FOOD REQUIREMENTS FOR THE GROWTH OF THE RAT

VII. AN EXPERIMENTAL STUDY OF INHERITANCE AS A FACTOR INFLUENCING FOOD UTILIZATION IN THE RAT[1]

H. P. MORRIS,[2] L. S. PALMER, AND CORNELIA KENNEDY

INTRODUCTION

In the literature concerned with the study of growth, where most frequently the albino rat has been used as the experimental subject, many data appear comparing two groups of animals often few in number and of indeterminate breeding. These animals had been grown on diets differing, presumably, in only one constituent. The food consumption of individual animals frequently was not obtained and more often was entirely ignored in the interpretation of the results. Even in cases in which food intake was considered, it was found difficult to dissociate the increased growth of the animals on a more complete diet from the factor of increased food intake that usually accompanied the greater growth.

It is generally recognized that as an animal actually increases in size its maintenance requirement increases, and any comparison of a larger animal on a complete diet with a smaller one on a restricted diet is considered practically impossible in arriving at a quantitative measure of the difference in the two diets. It is also known that in any given species individuals of the same sex exhibit a wide variation in the rate of growth and the mature size attained. That among such individuals there may be an equally wide divergence in the utilization of the food nutrients derived from an adequate dietary has been recognized recently by Palmer and Kennedy (1931).

They point out that the wide variations in the efficiency of food utilization that are present in laboratory animals of the same sex when consuming an identical dietary, not only complicate the interpretation of growth data obtained from experiments designed to measure the

[1] Part of the data contained in this paper was submitted by H. P. Morris to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1930.

[2] Portions of this investigation were carried out by H. P. Morris while holding a National Research Council Fellowship in Agriculture at the University of Minnesota Agricultural Experiment Station, 1930-31. He takes pleasure in expressing his appreciation for the facilities and equipment made available by the Division of Agricultural Biochemistry during the tenure of this fellowship, and for the advice and encouragement of Drs. R. A. Gortner, L. S. Palmer, Cornelia Kennedy, and other members of the faculty.

nutritative value of a single dietary variable, but often may lead to misleading or unwarranted conclusions. Their data do not give as many comparisons as are desirable between animals consuming exactly the same quantity of the same dietary, but they do show that the variations in efficiency of food utilization as measured by their efficiency quotient[3] method are of a magnitude great enough with small numbers of animals to vitiate conclusions when the differences in the effects of a natural compared with a synthetic diet are measured by size and body weight and the appearance and general condition of the animals.

In their investigations Palmer and Kennedy obtained data which indicated that line bred rats without admixture of outside blood for several years consuming equal quantities of the same diet exhibited wide variations in the amount of growth made as measured by increase in live weight. These wide differences in the gain in weight were encountered even when the experimental period included only that portion of the rat's life in which the most rapid growth takes place. It was imperative, therefore, that further attempts be made to evaluate the causes of these large variations in the efficiency of food utilization.

The most promising line of attack of the problem appeared to be an investigation to determine whether or not efficiency of food utilization is affected by heredity. It was believed that environmental conditions could be kept constant and so adjusted as to give the growing animals the maximum stimuli for growth, and, therefore, any heritable differences in efficiency of food utilization could be detected.

Up to the present time, studies of developmental processes have embraced almost exclusively the methods in which consideration is given only to controlled changes in the environment. The worker in the field of animal nutrition has built up a highly effective technic by which he studies the action of one set of variables comprising the environment while disregarding the effects of hereditary factors involved. To illustrate this point by a concrete example, the following statement is quoted from Blunt and Cowan (1930, p. 2): "The one variable with Sherman and Campbell was the food of their animals. Starting with rats from the same litter so that the heredity and early nutritive conditions were the same, they have kept their animals for generations, one family on a diet one-sixth whole milk powder and five-sixths ground whole wheat, and the other one-third whole milk powder and two-thirds ground whole wheat, both groups having also a little common salt. *The only difference between the two sets of rats is, therefore, the relative proportion of the milk and wheat which they have eaten.*"[4]

[3] Efficiency quotient = (digestible dry matter consumed ÷ gain in live weight) ÷ mean weight during the experiment) × 100.

[4] Italics are ours.

It is apparent that Blunt and Cowan believe that by using litter mates a sufficiently controlled and uniform material is obtainable for use in nutritional investigations. We know that this is not always the case. "Litter mates," says T. H. Morgan (1926), "may be as different, from a genetic point of view as any two other individuals of the race. While it is true that there may be a slightly better chance, if litter mates are used, of obtaining nearly similar individuals, unless animals have been long inbred, this sort of material is not sufficiently good for exact physiological work, whether that work is on embryos or on adults."

It is clear, therefore, that before attempting experimental work in which the environment is to be made the variable, care should be used to obtain animals as homozygous as possible in all genetic factors that may affect the interpretation of the results. It has been shown by Wright (1921) that it is theoretically possible after long continued inbreeding in mammals to secure very nearly genetically pure individuals. He showed further that sib matings were the most effective type of breeding to procure homozygosity in animals. As yet, however, this ideal genetically pure individual has not been produced. If it is possible to obtain experimental animals of greater uniformity of response, the precision of nutrition experiments will be greatly facilitated.

REVIEW OF THE LITERATURE

Differences in efficiency of food utilization have been recognized by physiologists since the early days of the use of the biological method in biochemical research. Thus Hopkins (1912) considered the cost of growth of his animals in terms of calories required for unit gain in body weight in order to explain the remarkable growth-promoting effects of small additions of milk to certain synthetic diets. Macallum (1919), using the same method of calculation, obtained data showing the energy cost of a gram of gain to be greater for female than male rats. Smith and Carey (1923) and Levine and Smith (1927) calculated food utilization by determining the calories required by rats to make a definite gain in weight. Male rats between the ages of 40 to 50 days were used in these experiments. The time required to gain 21 grams live weight, which was the experimental period they used, is obviously too short to give an accurate indication of the efficiency of food utilization. Somewhat later Levine and Smith determined the average calorie intake per day per 100 grams of live weight. They selected animals of the same initial body weight, which made the same gain in weight in the same time. Altho their longest period was the time required for the rats to gain 93 grams live weight, they produced data agreeing with

observations of Macallum (1919) that the calorie requirement diminishes with age.

The calories required per gram increase in weight were calculated by Griffith (1929, 1929a, 1929b, 1930) on groups of rats. It is not possible, therefore, to determine from his data the efficiency of food utilization for individual animals. Hitchcock (1927) used the same calculation in determining the energy requirement of the rat for growth and activity. Altho he did not take it into account in the interpretation of his data, he found that the cost of the increase in live weight was greater for the females than for the males.

Evvard, Nelson, and Sewell (1928) calculated the amount of food required to make 100 grams gain. This method has long been used in livestock feeding trials. It has the disadvantage that it neglects to give weight to increased maintenance cost of the larger animals.

Titus, McNally, and Hilberg (1930), in feeding trials with growing chicks, determined the average efficiency of the food for the different lots by calculating for two-week periods the gain in live weight per unit weight of food consumed. Their method was used in experiments in which individual food consumption records are difficult to obtain. They also studied the relation between average cumulative efficiency of the diets and age of the birds and found that except for a slight initial rise, there was a straight line relationship between these variables that decreased with age.

In work with mice, Thompson (1926) recognized individual differences in food utilization and capacity for growth. She determined the percentage of the food consumed that was retained as body weight. She found that the males had a higher percentage of food utilization than the females. Beard (1926) and Dawbarn (1928) determined the calories assimilated per unit of the 2/3 power of the body weight. Beard's data showed the same superior utilization of food by male over female mice as observed by Thompson, while Dawbarn's data showed marked individual differences in the efficiency index. In arriving at a method for determining the energy requirements of dogs, Cowgill (1923) (1928) calculated the calories consumed per kilo of body weight. His data showed considerable variation in the number of calories required by different dogs to maintain a constant body weight.

Mitchell and Carmen (1926) determined the basal metabolism, food consumption, and composition of gains in weight insofar as that was possible for male and female rats for a period comprising the major portion of the growth cycle. They arrived at a percentage utilization that may be expressed by the following formula:

$$\text{Percentage utilization of food energy} = 100 \times \frac{(\text{av. basal heat production} \times \text{days on the experiment}) + \text{energy in the gain}}{\text{metabolizable energy}}$$

These authors used average values because of the wide variation in the gain of the check rats that had been used to determine the initial composition of the test animals. They, also, determined the total heat production of the various test rats from average values; this procedure undoubtedly has a tendency to minimize the individual differences in efficiency of utilization of food energy that they obtained. They computed the basal heat production for maintenance of rats from an average of the basal metabolism observations of all the males and females because the sex of the animals on maintenance had not been noted. Disregarding the possible errors introduced when determining the percentage utilization of energy for maintenance by Mitchell and Carmen's method, the data indicated a very constant value for the cost of maintaining their rats over a considerable range in body weight and that by far the greatest proportion of food energy was used for maintenance. The maintenance values ranging from 91.3% to 96.8% are much more uniform than the values ranging from 62 to 81 which they obtained for the growing animals. Mitchell and Carmen also state that "no clear sex differences in the composition of the gains are evident, although for each litter of rats, except litter A, the highest percentage of ether extract and the highest quantity of energy per gram of gain was exhibited by one of the two females in the litter."

Mitchell and Carmen's method is slow, laborious, and not suitable for use in the laboratory where animals are wanted for breeding or other experiments following a determination of their efficiency indices. In the method for calculating efficiency of food utilization that was developed by Palmer and Kennedy (1929), decided differences were observed in efficiency of food utilization in individual rats as measured by

$$\frac{\text{dry matter consumed}}{\text{grams gain in weight}} \div 100 \text{ grams body weight (mean weight for the period)}.$$

The experimental period included 6 weeks beginning when the rat reached 60 grams live weight. Decided differences were noted between the sexes, the index values for the male rats being much less than for the female rats. The method of calculation used takes into consideration the size or mass of tissue of the animal and, therefore, gives added weight to the maintenance requirement. Obviously, as was pointed out above, the maintenance is a factor of major importance in any consideration of the efficiency of food utilization, since by far the greater

proportion of the food energy is expended in maintenance. The expression of an efficiency index in terms of dry matter, when the diets to be compared are isodynamic, has the advantage of being more readily determined than when expressed on a calorie basis. In the efficiency index method advocated by Palmer and Kennedy the cost of dry matter consumed per unit gain in weight is determined for each animal. The ratio of dry matter ÷ gain in weight is then calculated in relation to the total size of the animal, i.e., the mean weight for the period. This latter calculation holds only during a period when the growth is essentially a linear function of time. If the efficiency of food utilization is studied over a longer period, when growth is not linear, it would be essential to make determinations of the efficiency index for relatively short intervals of the experimental period, unless it were possible to obtain the average mass of tissue of the animal in some other manner for the entire period.

On the other hand, efficiency indices may be calculated according to this method when growth is not linear if the length of the periods is the same and the age of the animals is also the same. The index thus obtained can be used only in comparing the differences between the linear and non-linear growth, since the mean weight of the animal in the latter case would not be an accurate measure of its vital tissue.

THE PROBLEM AND GENERAL METHOD OF PROCEDURE

This study had three major phases. First, a large F_2 population was produced from the inbreeding of F_1 progeny in order to study the segregation of the second generation hybrids to determine whether or not they followed a Mendelian population. Second, animals were selected from an F_2 population which showed either a high or a low efficiency. Those of high efficiency were mated together in an attempt to produce offspring of high efficiency, and similarly those of low efficiency were mated together in an attempt to produce a strain of low efficiency animals. Selection and inbreeding were practiced in succeeding generations in the hope that strains would be developed which would breed more or less true for the two general levels of efficiency. Third, chemical analyses were made on a number of animals having different efficiency indices. In addition to the chemical analyses, measurements were made of the small and large intestines, and of the caecum. By a study of these data valuable information has been obtained to help explain some of the causes of the previously observed sex differences in the efficiency indices.

The ultimate aim of this research is to obtain a greater degree of uniformity in the utilization of food by rats that are to be used in

nutrition experiments, when the diet is to be made the variable. It is believed that the results obtained in these studies represent only a beginning in the attempt to reach that objective.

The ration and its preparation.—In order to demonstrate the presence or absence of heritable factors influencing the efficiency of food utilization, it is necessary to control environmental conditions. First, the ration should be optimum in all respects for growth. Second, animals should be kept under ideal and uniform conditions throughout the experimental period. Inasmuch as all the animals in this study could not be placed on experiment at the same time, it was necessary insofar as possible to maintain the same laboratory conditions throughout the duration of the investigation.

The experiments that are to be described deal primarily with the variations in the economy of growth of rats of the same initial body weight and of approximately the same age when kept on the same diet for the same length of time. It was therefore of great importance to select a diet that would give the animals every known dietary growth stimulus. The diet chosen was a modification of one that Osborne and Mendel (1926) had reported as giving great acceleration to the rate of growth in the rat. The basal portion of their diet consisted of the following constituents:

	Per cent
Casein	35
Starch	37
Lard	15
Butterfat	9
Salt mixture	4

In this investigation the above basal diet was modified by using 36.5% tapioco dextrin and 3.7% McCollum's (1916) salt mixture No. 185 plus 0.8% $CaCO_3$ (Palmer and Kennedy 1927) in place of Osborne and Mendel's 37% starch and 4% salt mixture. The tapioca dextrin was made by autoclaving tapioca at 16 pounds pressure over night with the steam admitted to the pressure chamber. After autoclaving, the dextrin was dried and ground. Commercial casein and Swift's "Silverleaf" brand lard were used. The butterfat was prepared from sweet cream butter purchased from the Land-o-Lakes Creameries Association. This butter was packed in one-pound sealed export containers. It was from the same pack produced in July from cream obtained from animals on flush summer pasture. After purchase it was kept in a room held at a constant temperature of —17° C. One or two pounds were removed at a time, melted, filtered at 50–60° C. and then kept in a refrigerator until used.

Osborne and Mendel fed their basal diet *ad libitum* and gave each animal a daily supplement of yeast, fresh liver, and dried lettuce. In this work the basal diet was fed *ad libitum*. From preliminary trials reported by Palmer and Kennedy (1931), it was found that the optimum quantity of daily supplements per animal would be provided by 20 grams of fresh lettuce, 0.5 gram of fresh liver, and 0.25 gram of dry brewer's yeast. According to Palmer and Kennedy larger quantities of these supplements gave no greater growth and seemed to decrease the digestibility of the dry matter of the ration.

Fresh head lettuce was purchased from the University cold storage plant. In order to insure a minimum variation in moisture content of this supplement, it was either wrapped in wet newsprint paper and placed in a refrigerator as soon as it was received from cold storage or put in an air-tight container in a Frigidaire. The daily portion of lettuce for each animal was weighed separately on a torsion balance to an accuracy of 0.05 gram. Frequently chemical determinations on the lettuce showed very little variation in its moisture content. Since the animals were fed lettuce for 42 consecutive days, slight variations from day to day would introduce no appreciable error in the determination of the total dry matter consumed. For these reasons only one factor was used throughout the investigation to convert the fresh lettuce into dry matter. Occasionally, usually during the first few days of the experiment, a few of the rats would not consume all of the 20 grams of fresh lettuce. That portion of lettuce remaining was weighed, and later when the rat was consuming all of its daily supplement of lettuce, it was given one or two grams additional fresh lettuce until its total consumption of fresh lettuce had been brought back to normal. These additional portions seldom amounted to more than one or two grams of dry matter.

Fresh beef liver was secured in lots of from 4 to 6 pounds. This meat was cut up into pieces of approximately 8 ounces and placed in an air-tight can in a room held at a constant temperature of —17° C. One of these pieces was removed at a time and placed in the icing chamber of a Frigidaire. It was thus kept in a tight container in a frozen condition at all times, which obviously is important from the standpoint of moisture loss. Before feeding and while still frozen this liver was cut into small squares of approximately 0.5 gram. It was then weighed immediately on a torsion balance to an accuracy of less than 0.05 gram.

Dry whole yeast[4] was used throughout these experiments. Tablets

[4] Two brands of pure dry yeast have been used, that manufactured by the Northwestern Yeast Co., Chicago, Ill., and Fleischmann's pure dry yeast, manufactured by Standard Brands, Inc., New York City.

weighing 0.25 gram each were made, and each rat was fed one tablet daily.

The basal ration was mixed weekly, and enough of its was weighed out for each rat to last approximately a week. The extra basal diet was kept in an ice box and was used as needed. The feed dishes were washed each week.

Care of the animals.—Insofar as possible the animals were kept at the same environmental temperature throughout the course of these experiments. The temperature of the rat room was thermostatically controlled. At first the thermostat was set for a temperature of 74° F. The temperature maintained was not absolutely uniform for all of the 24 hours of the day, and, consequently, in October, 1929, connections to the high pressure steam line were installed on some of the radiators. This improvement insured available steam pressure at all times, and decreased very materially the previous variations in the room temperature. Experiments of Goto (1923), and Benedict and MacLeod (1929) indicated that the critical temperature of the fasting rat is near 28° C. While a rat consuming large quantities of food would have a much lower critical temperature than while fasting, it was thought best to raise the room temperature to 79° F. in order to lessen the possibility of the rat using unnecessary energy merely to maintain its normal body temperature. Since October, 1929, the temperature of the laboratory has been maintained as nearly as possible at 79° F. The maintenance of a uniform environmental temperature is emphasized because, as Benedict and MacLeod (1929) have shown, the rat is very sensitive to changes in temperature, and the consumption of food bears a close relation to the laboratory temperature. This point was of considerable importance in view of the fact that the rats were kept on wire screens in individual metal cages without any nesting material, conditions which would favor rapid heat losses from the body.

The rats were weighed weekly, the weighings being done in the morning before the feeding of the supplements in order to minimize as much as possible the unavoidable errors due to the presence of greater or lesser quantities of undigested matter in the alimentary tract. All weights were made on a Toledo balance graduated for differences of two grams but easily read to an accuracy of one gram. A feed cup of the McCollum type was used. It allowed for a very accurate record of food consumption especially with this basal diet, which is high in fat. The basal food consumption was obtained by difference. The diet packed readily in the food container, and the rats rarely scattered their food. Frequent observations were made of the shavings underneath the wire screen for evidences of food wastage. Whenever a weighable

quantity was observed, it was taken into consideration in the calculations of the weekly food consumption. Distilled water containing a trace of iodine was before the animals at all times. Each week the cages were washed and then sterilized by live steam.

At the end of the sixth week the rats selected for breeding purposes were taken to the stock-animal room and mated. All matings were made between brother and sister. When the animals were mated they were placed on the regular breeding colony diet made up of a mixture of natural foods. This diet has been in use in this laboratory for several years and has given entirely satisfactory results for maintenance, growth, reproduction, and lactation. This is an important point because it is not known whether the experimental diet with its high content of fat and protein would prove adequate in all these respects if animals were kept on it for several generations. In addition to the regular stock diet, the breeding rats used in this experiment were given fresh lettuce several times each week.

Sampling and analytical technic.—At the close of the six weeks experimental period some of the rats not used for matings were autopsied and their intestines and caecum measured by means of a meter stick and a ruler graduated in centimeters. Care was taken to avoid any more stretching than necessary. The alimentary canal of each rat to be analyzed was emptied of its unabsorbed food residues. The animal was then put in a glass Mason jar which was tightly sealed and was placed in a cold storage room held at a temperature of —17° C. After it had become frozen the carcass was put through an ordinary meat chopper several times, or until it was thoroly minced. The grinding was done in this low temperature room to avoid, insofar as possible, moisture losses in handling. The minced tissue was then returned to the same jar. It was kept at this low temperature until the chemical analyses were started. It was brought to the laboratory the night before it was to be sampled in order that it might thaw and come to room temperature. Just before weighing the sample for analyses, it was thoroly mixed in the original jar by means of a long porcelain spatula. Analyses were run in duplicate from samples that had been weighed by difference and placed in lead caps. The dry matter was determined by keeping these samples five hours in a vacuum oven held at a temperature of 105° C. The moisture-free residue was placed in paper extraction thimbles by slitting the edges of the lead cap and folding it together but leaving an opening at the top to allow free dripping of the ether on the sample. These samples were then placed in Soxhlet siphon extraction tubes and were extracted with ether continously for 36 hours. Both the moisture and the fat-free residue, and the extracted

residue were held in an air oven at 105° C. for 2 hours, or until constant in weight. In this way it was possible to get a double check on the percentage of ether extractives in the various rats analyzed. The nitrogen was determined by the usual Kjeldahl method and the factor 6.25 was used to convert the nitrogen to protein. The ash was determined from the residue remaining after the ether extraction. It may be slightly low if the ether extract contained an appreciable amount of phospholipins. For the comparative purposes for which the analyses were made, it is believed this procedure introduced no appreciable error.

EXPERIMENTAL

Determination of the efficiency index.—The average percentage of dry matter in the various portions of the experimental diet was as follows:

	Per cent
Basal diet	93.9
Fresh head lettuce	4.3
Fresh beef liver	29.7
Dried yeast	95.5

The total dry matter contained in the supplements amounted to 8.7 grams per week. This value was used in calculating the efficiency indices of all the rats studied. Undoubtedly some variations occurred in the dry matter as thus determined, but such variations were not great enough to affect the efficiency index to any appreciable extent. This efficiency index was calculated according to the method recommended by Palmer and Kennedy (1929) except that the total dry matter consumed was used instead of the digestible dry matter. The calculations thus determined are expressed as follows: efficiency quotient = (total dry matter consumed ÷ gain in live weight) ÷ mean weight of animal for the period × 100. Since the amount of the supplements was kept constant, and as Palmer and Kennedy (1931) have shown that in rats there is very little variation in the digestibility of this diet, much less than would be necessary to account for the wide differences in efficiency of food utilization, it was believed unnecessary to correct the calculations for this factor in determining the efficiency of food utilization as expressed by the efficiency index method.

In the efficiency index method, the larger the value obtained the less efficient is the food utilization of the individual it represents. It will be recalled, also, that the efficiency index is calculated for animals during the period when they are making the most rapid growth. The growth during this period is essentially a linear function of time, and therefore justifies the use of the mean weight for determining the

average size of the animal for the period. In most cases the experimental period of six weeks ended by the time the rat was ten weeks old. The efficiency indices as calculated include an evaluation of the cost of maintenance as well as the cost of growth. Because of the wide difference, however, in efficiency quotients of the two sexes, it was impossible to compare them on the same scale unless a correction factor was introduced and applied to all the individuals of one sex to bring them on to a scale comparable to those of the other sex. Such a factor was obtained early in the investigation by dividing the average efficiency quotient of 83 F_2 females (2.249) by the average efficiency quotient of 90 F_2 males (1.340). The factor thus obtained was 1.678, and it has been used throughout this investigation to raise the efficiency indices of the males to a scale comparable to that of the females. Such a procedure is not absolutely accurate even for the comparisons made herein because it does not take into consideration the entire population compared. Sufficient numbers were used in obtaining this factor, however, to give a reliable ratio between the efficiency indices of the two sexes. The use of the factor thus obtained, in bringing the male efficiency to the same scale as the female, introduces no appreciable error in the comparisons made. This point is mentioned here to indicate that the factor obtained is not an absolute value. It might be different for animals of another strain raised in this or other laboratories and affected by different hereditary as well as environmental conditions. It is also probable that the ratio may vary between the two sexes within any given litter. Such variation would be due largely to insufficient numbers of rats in any one litter.

Efficiency of Food Utilization by Generation and Family

Figure 1 shows the results of several years' study of the influence of breeding and selection upon the efficiency of food utilization for growth. The pair of animals from which all the rats shown in this figure are descendent, and which are designated as the P_1 generation, was not closely related. No information, however, was available regarding the variation in efficiency of food utilization of the parental strains. It was deemed inadvisable to wait to begin this study until strains of animals were obtained that were fairly uniform for different degrees of efficiency. In fact, when the study was first begun it was not even known whether it was possible to influence the efficiency of an animal by breeding and selection. The P_1 female was homozygous for the hooded color pattern; the P_1 male was an albino. All the F_1 individuals, therefore, were hooded, and three-fourths of the F_2 generation were hooded. Accurate records were kept of these color pat-

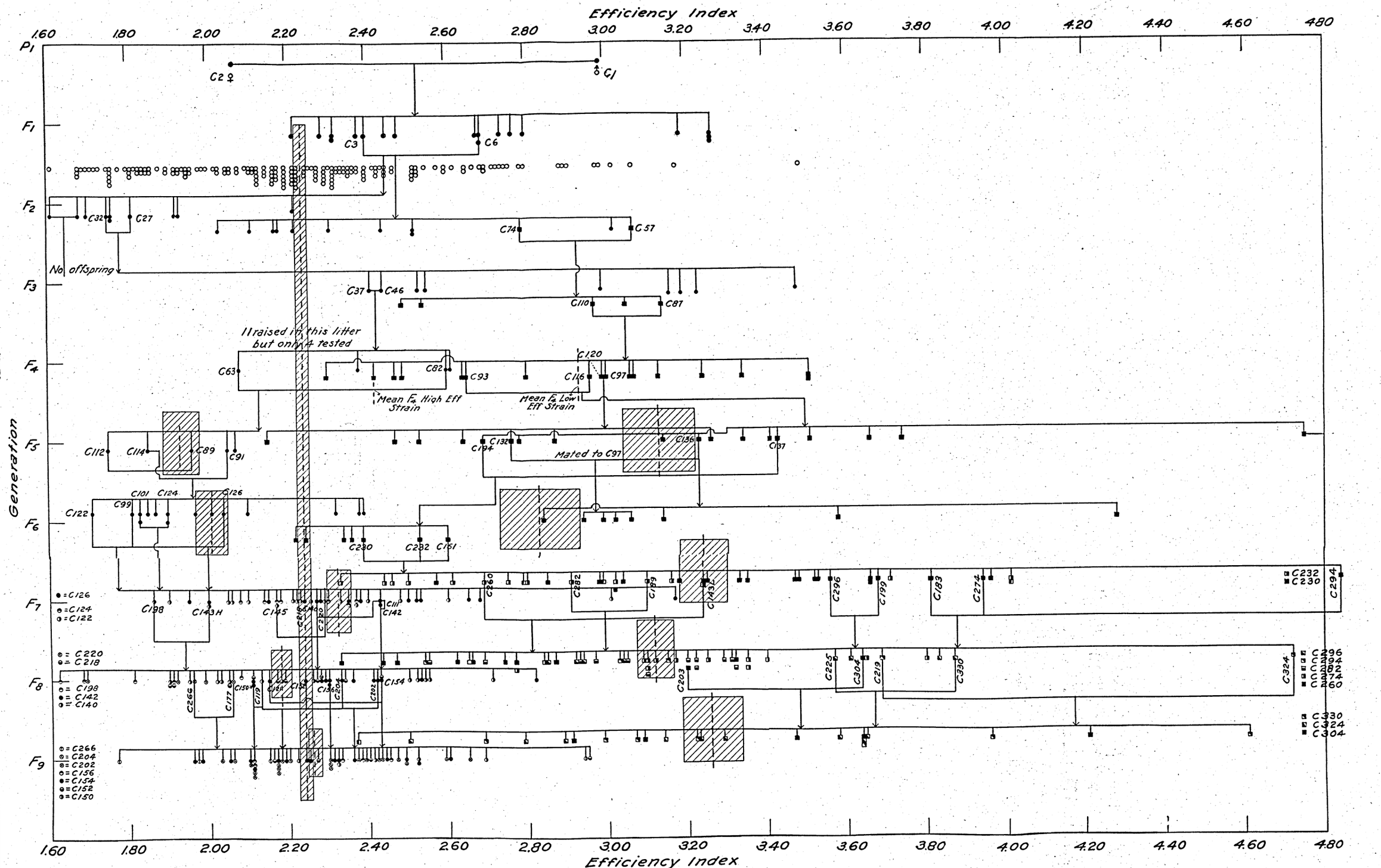
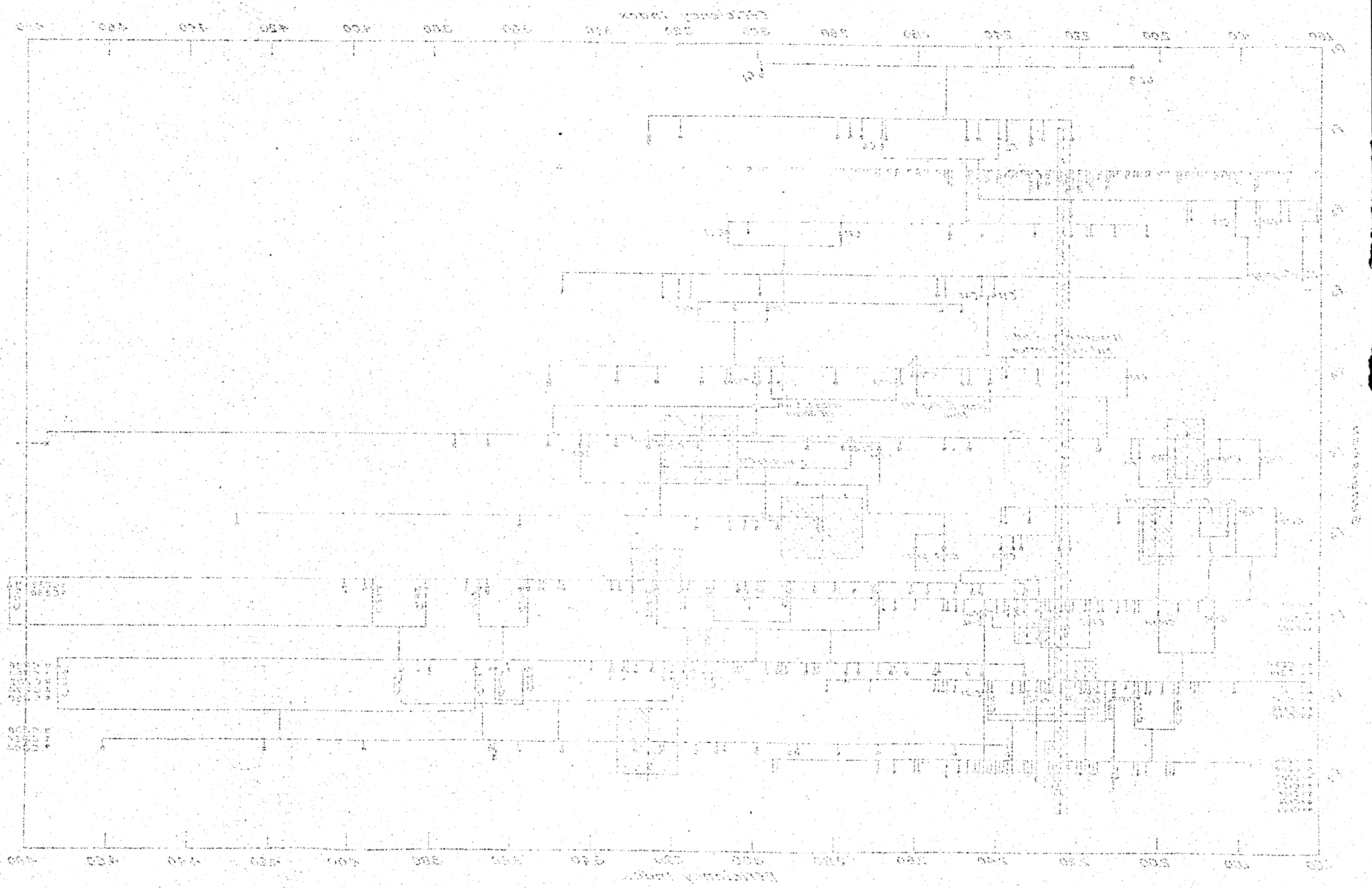


Fig. 1. Inheritance of efficiency index from parent (P_1, F_2) animals together with that of two inbred descendent families, selected for high and low efficiency.



terns in the hope that some genetic relationship would be found between color pattern and efficiency. No difference was discovered during the development of the high efficiency strain into the F_9 generation and the low efficiency strain into the F_6 generation.

During the continuation of the high and low efficiency strains to F_{14} and F_9 in the high and low efficiency strains, respectively, a marked and almost uniform difference in color pattern appeared. Both strains have white tails, except for a very short length of black at the base, but the high efficiency strain now has a uniformly broad stripe of fairly uniform width extending from the head to the tail, both hind quarters being frequently entirely black. The low-efficiency strain, on the other hand, has a less wide and more irregular black marking on the back with a marked tendency to be narrow or to break completely at the neck between the hood and the markings on the back. This difference between the two strains is now sufficiently uniform that there is little or no difficulty in selecting by inspection the strain to which an individual belongs. The difference now noticed is evidently traceable to the fact that all the individuals left in the high efficiency strain are descendants of one pair of F_5 rats which undoubtedly carried the factors for the color pattern that now characterizes this strain. Similarly, all of the low efficiency animals are descendants of one pair of F_3 animals, which evidently determined the color pattern now predominating in the strain. Both of these pairs are descendants of the same F_1 pair out of P_1.

Figure 1 shows the distribution according to the efficiency indices of all the rats descending from the P_1 animals for the F_1 and F_2 generations, but not all the animals produced in subsequent generations. The figure is arranged according to generation, efficiency, and frequency. Each dot, square, or circle represents the efficiency index of one animal. Where there are two or more animals in any given generation having the same efficiency quotient it is so represented by similar symbols arranged in a verticle order on the figure. The ordinate, therefore, not only represents the generation but also the frequency of animals in any given generation.

Turning now to the F_1 population, it will be observed that most of the individuals are intermediate when compared to their parents altho the variability in efficiency of food utilization of the parent stocks is not known. All the F_2 individuals produced by various combinations of matings among the F_1's and represented on Figure 1 by circles show a considerably wider range in the efficiency index than is represented by the two parental strains. Since it is not known how uniform were the parent strains for high and low efficiency, it may well be that some

of the variations obtained in the F_2 population were due to the heterozygosity of the parental strains. It is possible, however, that this wide spread in efficiency indices may indicate the action of multiple factors on the efficiency of food utilization in the rat.

Since the testing of 175 individuals in the F_2 generation for efficiency of food utilization did not yield any clear-cut evidence of the segregation of Mendelian factors, it was decided to try selective sib matings to see if the efficiency of food utilization could be altered in subsequent generations. To this end, several of the most efficient animals of the second generation were mated together, brother to sister, while several of the animals poorest in efficiency of food utilization were mated by the same system of mating. The second generation, in Figure 1, shows two such matings of the highly efficient animals and one mating of the poorest pair. The descendants from the particular matings, shown in Figure 1, illustrate the objective of the experiment, namely, the study of the animals in each generation of the descendants of ♀ C 74 mated to ♂ C 57 in the less efficient group and ♀ C 22 mated to ♂ C 27 in the more efficient group. The two litters in the F_2 generation from which these two pairs were selected are represented twice in Figure 1, i.e., by solid dots and also by the circles in the total F_2 population. The descendants of one other pair of highly efficient animals of the F_3 generation were studied to the F_7 generation but the family was then discontinued because of their slower breeding capacity. The animals selected for the lower efficiency group (higher index value) have been designated in Figure 1 by solid or partly solid squares; the animals in the more efficient line have been designated by solid dots through the sixth generation and from then on by solid, half solid dot-like symbols, and by circles containing various configurations. Near the margins will be found the legends for the symbols indicating the litters produced by the different dams for each of the later generations. This system makes it possible to follow the family tree for any particular litter. Several litters were raised in each generation in order to obtain enough animals to make a statistical study of the data.

The results of nine generations of selective inbreeding for the two general levels of efficiency of food utilization, as illustrated in Figure 1, show that definite hereditary factors do affect this efficiency. The average differences in food utilization for the different generations of the two strains are brought out in Table 1, and in Figure 1 are indicated by heavy broken vertical lines. If the F_5 generation is left out of consideration because of the very limited number of animals in the high efficiency strain, it is seen that the difference between the means has increased from 0.82 to 1.00 in the last four generations con-

sidered in this paper. Table 1 shows, as indicated by these differences, that the low efficiency strain is, on the average, about 40 per cent less efficient that the high efficiency strain. To obtain a measure of the significance of these apparently definite average differences the probable errors of the mean efficiency indices for each generation have been calculated when enough animals were available. The probable error is designated by the cross-hatched area on either side of the heavy line representing the mean efficiency quotient. The heavy line from the F_2 generation to the top of the chart is the average efficiency index for the second generation. The probable error for this mean is smaller in comparison with the probable errors for the other means, not because there was a lower range of variability but because of the larger number of observations made in this generation in contrast to the later generations.

The family selected for poor efficiency of food utilization shows a very definite lower efficiency than the mean efficiency for the F_2 population. There is also indication that the efficiency of food utilization of this family is decreasing; at least the spread between the two families is definitely increased from the sixth to the ninth generations. Another very significant point to be observed in Figure 1 is the very few instances in which an animal in the poor efficiency family has made as efficient utilization of food as the average for the F_2 generation. There have been only two such animals and they are only slightly more efficient than the second generation average. There have been no instances of this since the sixth generation. It becomes, therefore, apparent from this figure that a strain of rats has been established that is very definitely below the average in efficiency of food utilization; and, further, that this strain reproduces animals of a similarly low efficiency. However, this would not be a good strain to try to maintain as a breeding stock from which to produce experimental animals for nutrition investigations because of the very wide range in the efficiency of food utilization of the individuals in the family. The poor breeding qualities of the animals in this family in the early generations were responsible for the fewer generations we were able to obtain during the same time required to produce several additional generations in the strain selected for higher efficiency.

In selecting animals for the high efficiency line, the very efficient animals were mated together each time, and while the third and the fourth generations did not reproduce at all uniformly for high efficiency of food utilization, from the fifth generation to date the animals produced have been uniformly efficient in food utilization at a level approximately the same as the F_2 average. The fact that in this family

there have been obtained through several generations groups of animals with high efficiency of food utilization is of greater significance than the fact that a family of animals was produced having poor efficiency, because it will be recalled that only sib matings have been practiced, and Figure 1 shows quite definitely that there has been no deterioration in this family insofar as their efficiency of food utilization is concerned.

The seventh, eighth, and ninth generations of the high efficiency strain showed a fairly uniform average efficiency of food utilization. This may indicate that this family has become fixed insofar as their efficiency of food utilization is concerned. According to Sewall Wright (1921) animals produced by sib matings theoretically should have become about 90% homozygous after nine generations of inbreeding. If, then, these animals have become more nearly homozygous, we should expect a more uniform average efficiency of food utilization and also less variability. It can not be stated definitely, however, that the variability would be less for a population as large as that shown for the F_2 generation. Every indication points to approximately the same variability because a few animals in the seventh, eighth, and ninth generations were as inefficient as the least efficient animals in the second generation. The efficiency of food utilization of this group, however, is much less variable than the efficiency of food utilization for the poor efficiency group. This is illustrated by the probable errors of the mean efficiency indices of the two groups.

Sex Difference in the Efficiency Quotient

The difference in the efficiency indices between the sexes has been studied simultaneously with the selective inbreeding. Weekly weights of the animals and their food consumption have been made, thereby giving data on which the efficiency index could be calculated for periods of less than six weeks in length. These data have been analyzed to obtain information regarding the most logical period for making an efficiency determination.

Table 2 illustrates the average difference in the indices as calculated for the periods from one to six weeks in length. The efficiency quotients for the first 277 rats studied are included in these statistics. According to these figures, the general trend of the male efficiency index value is downward and that of the female is upward. It will be recalled that the more efficient individual has a lower index value. This means that the two sexes are more nearly alike at the end of the first week than at any of the succeeding weeks. Table 2 also shows that the ratio between the efficiency indices of the sexes increases at a practically constant rate as the length of the period increases. The variability of

the efficiency index decreases for the male with each week increase in the length of the period. The longer period, at least up to six weeks, gives a better indication of the efficiency index of the male than a shorter period and, as indicated by the probable error, is less subject to errors of random sampling. The converse is true for the efficiency index for the female. Here the least variable period is the first week and the most variable is the six weeks.

Since it has been shown (Mitchell and Carmen 1926a) that the largest part of the energy consumption of growing rats is dissipated in basal heat production, and since it is also known that the basal heat production is more nearly related to body surface than to body weight, it was thought advisable to compare the efficiency indices calculated per unit of surface area with those calculated per unit of body weight.

Efficiency of Food Utilization per Unit of Surface Area

The surface area has been calculated from the mean weight of the rat for each of the periods studied by use of the Meeh type of surface area formula as modified by Lee and Clark (1929), namely, $S = K\ wt.^{0.60}$.

where S = surface area in sq. cm.
K = constant
wt. = body weight in gm.

The constants for K, which Lee and Clark obtained experimentally, are 12.62 for males and 12.44 for females. The weight of the rat was raised to the 0.60 power and then multiplied by the respective value of K. The resulting product gives the surface area in sq. cm. Lee and Clark's formula was used because it gives results that probably represent the most accurate method of calculating the surface area of the rat without actual measurement of each individual.

In Table 3 are presented data for the average efficiency quotients as calculated to both 100 grams of body weight and 100 sq. cm. of surface area. The results of these calculations are shown graphically in Figure 2.

The efficiency index of the female, which is practically constant for each period when it is calculated on the body weight basis, rises in a straight line when it is expressed per unit of surface area. The efficiency index for the male, on the other hand, gives a straight horizontal line when it is expressed on a surface area basis, and a curve decreasing with length of the period when it is expressed per unit of live weight. The question arises as to which method is most accurate for expressing the efficiency of food utilization.

Table 1

Differences Between the Efficiency Indices of the Two Strains in the Generations F_5 to F_9 Inclusive

Generation	High efficiency strain		Low efficiency strain		Difference of means	
	N	Mean p.e.	N	Mean p.e.	Actual	Percentage*
F_5	5	1.94 ± .043	18	3.13 ± .090	1.19	61.4
F_6	14	2.01 ± .040	15	2.83 ± .100	0.82	40.8
F_7	41	2.32 ± .026	47	3.24 ± .043	0.92	39.6
F_8	48	2.18 ± .026	41	3.12 ± .058	0.94	43.1
F_9	56	2.26 ± .021	21	3.26 ± .075	1.00	44.3

* Compared with the index values for the high efficiency strain.

Table 2

A Study of the Efficiency Index of 146 Male Rats and 141 Female Rats for Experimental Periods Varying in Length from 1 to 6 Weeks

The periods are cumulative, i.e., all previous periods are included in the next period.

Length of period	Mean efficiency index for 146 male rats	Standard deviation for male rats	Mean efficiency index for 141 female rats	Standard deviation for female rats	♀ Efficiency index ÷ ♂ Efficiency index
weeks					
1	2.027 ± .027	0.482 ± .019	2.218 ± .022	0.376 ± .016	1.095
2	1.803 ± .022	.393 ± .0155	2.209 ± .023	.382 ± .016	1.226
3	1.634 ± .016	.289 ± .011	2.2497 ± .023	.391 ± .016	1.377
4	1.473 ± .015	.267 ± .011	2.256 ± .025	.422 ± .018	1.532
5	1.401 ± .013	.239 ± .0094	2.2796 ± .026	.434 ± .018	1.627
6	1.383 ± .012	.221 ± .0087	2.3600 ± .026	.448 ± .019	1.706

Table 3

A Comparison of the Average Efficiency Quotient for 146 Male and 131 Female Rats When Calculated per 100 Grams Live Weight or per 100 sq. cm. Body Surface*

Length of period	Average dry matter consumed	Average gain in weight	Average mean live weight	Average mean surface area	Average dry matter / Average gain	Average efficiency quotient: Per 100 gm. body weight	Average efficiency quotient: Per 100 sq. cm. body surface	Average female efficiency index ÷ Average male efficiency index: Per 100 gm. body wt.	Average female efficiency index ÷ Average male efficiency index: Per 100 sq. em. surf. area
week	grams	grams	grams	sq. cm.					
				146 males					
1	55.08	35.43	76.70	170.6	1.554	2.03	0.91	....	....
2	123.27	72.56	94.25	193.0	1.699	1.80	.88	....	....
3	200.36	108.95	112.57	214.8	1.839	1.63	.86	....	....
4	282.02	146.35	130.85	235.0	1.927	1.47	.82	....	....
5	366.05	178.03	146.93	251.9	2.059	1.41	.82	....	....
6	452.22	204.52	159.88	265.1	2.211	1.38	.83	....	....
				131 females					
1	51.94	30.66	76.36	167.7	1.694	2.22	1.01	1.095	1.109
2	114.60	57.94	89.52	184.5	1.978	2.21	1.07	1.226	1.216
3	181.84	80.31	100.65	197.9	2.264	2.25	1.14	1.377	1.325
4	250.31	100.67	110.22	209.0	2.486	2.26	1.19	1.532	1.451
5	318.10	117.84	118.42	218.2	2.699	2.28	1.24	1.627	1.512
6	388.83	131.37	125.42	225.8	2.960	2.36	1.31	1.706	1.578

* Surface area calculated by use of the following formula: $S = K \text{ mean weight}^{.60}$
In which S = surface area in sq. cm., K = 12.62 for males and 12.44 for females.

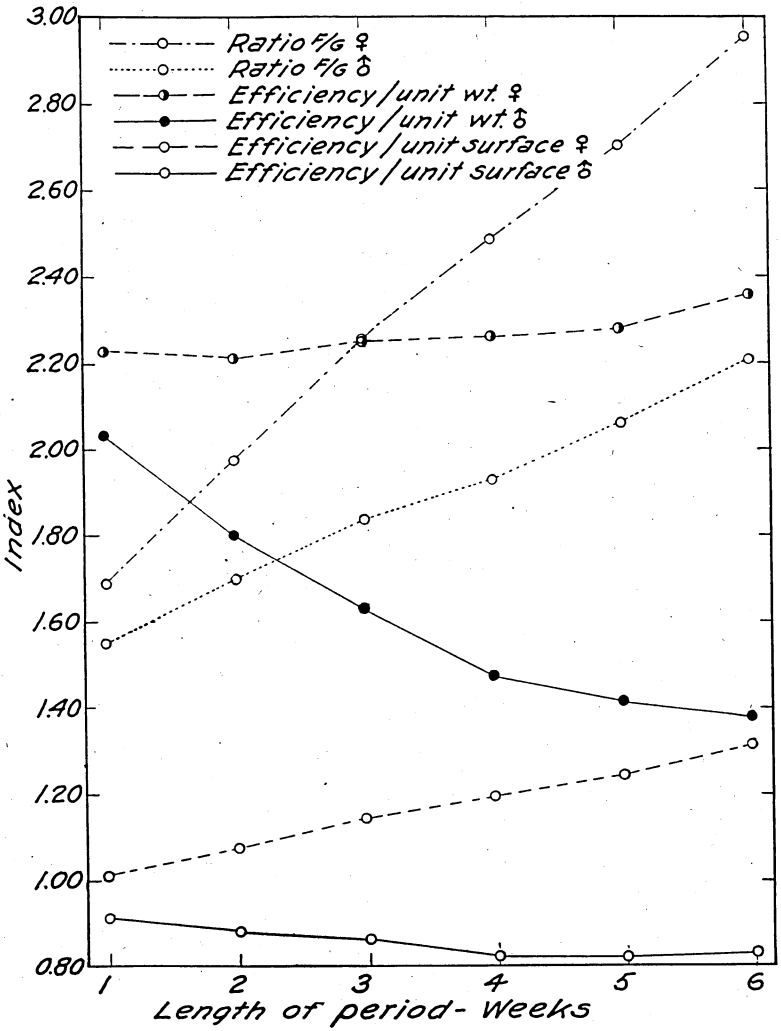


Fig. 2. Relation between the ratio of dry matter consumed per unit gain in weight for male and female rats and their efficiency indices when calculated per unit body weight (100 grams) and per unit body surface (100 sq.cm.) for periods of different lengths. Average figures for data on 146 male and 141 female rats on identical diet. Each longer period includes all periods previous to it.

The efficiency index curve for either sex in Figure 2, when calculated per unit of surface area, follows more closely the curve of dry matter consumed per unit gain in weight (F/G) than does the efficiency index curve expressed per unit of body weight. Hence it appears that the efficiency of food utilization is more closely associated with the surface area than it is with the body weight, a finding not inconsistent with the known fact that the largest part of the food, even

Table 4

Chemical Composition of Rats Arranged in Order of Decreasing Efficiency of Food Utilization as Indicated by the Efficiency Index

Female value minus male value = difference

	♀ 99	♂ 50	♀ 98	♂ 42	♀ 63	♂ 37	♀ 102	♂ 95	♀ 55	♂ 48	♀ 106	♂ 45
Efficiency index	1.69	1.05	1.71	1.08	1.76	1.10	1.90	1.10	1.94	1.11	2.08	1.29
♀ — ♂ values = Diff.	+0.64	....	+0.63	....	+0.66	....	+0.80	....	+0.83	....	+0.79	....
Dry matter %	43.78	37.51	43.47	37.59	41.52	39.48	41.06	39.42	44.88	37.55	44.83	36.87
Diff.	+6.27	....	+5.88	....	+3.04	....	+1.64	....	+7.33	....	+7.96	....
Nitrogen %	2.88	3.34	2.90	3.28	2.98	3.23	3.12	3.30	2.86	3.21	2.89	3.26
Diff.	−0.46	....	−0.38	....	−0.25	....	−0.18	....	−0.35	....	−0.37	....
Protein (N × 6.25) %	18.00	20.88	18.13	20.50	18.63	20.19	19.50	20.62	17.87	20.53	18.06	20.37
Diff.	−2.88	....	−2.37	....	−1.56	....	−1.12	....	−2.66	....	−2.31	....
Fat (Ether extract) %	21.98	14.08	21.98	13.98	19.95	16.66	18.34	16.53	23.37	14.36	23.64	13.47
Diff.	+7.90	....	+8.00	....	+3.24	....	+1.81	....	+7.01	....	+10.17	....
Fat-free dry matter %	21.80	23.43	21.49	23.61	21.57	22.82	22.72	23.89	21.51	23.19	21.19	23.40
Diff.	−1.63	....	−2.12	....	−1.25	....	−1.17	....	−1.68	....	−2.21	....
Ash %	3.56	3.30	3.38	3.37	3.20	3.25	3.78	3.05	3.49	2.88	3.73	3.34
Diff.	+0.26	....	+0.01	....	−0.05	....	+0.73	....	+0.61	....	+0.39	....
Final live wt., gm.	219	298	232	303	230	299	208	296	212	288	200	261
Diff.	−79	....	−71	....	−69	....	−88	....	−76	....	−61	....
Final fat-free wt., gm.	171	256	181	261	185	249	170	247	162	255	153	226
Diff.	−85	....	−80	....	−64	....	−77	....	−93	....	−73	....
Total fat, gm.	48	42	51	42	45	50	30	49	50	33	47	35
Diff.	+6	....	+9	....	−5	....	−19	....	+17	....	+12	....

Table 4—Continued

Chemical Composition of Rats Arranged in Order of Decreasing Efficiency of Food Utilization as Indicated by the Efficiency Index
Female value minus male value = difference

	♀ 18	♂ 130	♀ 114	♂ 26	♀ 41	♂ 113	♀ 100	♂ 291	♀ 119	♂ 122	♀ 288	♂ 290
Efficiency index	2.20	1.30	2.25	1.31	2.26	1.32	2.30	1.48	2.36	1.42	2.55	1.42
♀ − ♂ values = Diff.	+0.90	....	+0.94	....	+0.94	....	+0.92	....	+0.94	....	+1.13	....
Dry matter %	44.24	39.42	42.11	35.24	37.21	35.39	41.86	37.53	41.24	38.80	38.45	38.17
Diff.	+4.82	....	+6.87	....	+1.82	....	+4.33	....	+5.44	....	+0.82	....
Nitrogen %	2.98	3.11	3.26	3.33	3.16	3.30	3.14	3.27	3.04	3.45	3.22	3.27
Diff.	−0.13	....	−0.07	....	−0.14	....	−0.13	....	−0.41	....	−0.05	——
Protein (N × 6.25) %	18.63	19.44	20.37	20.81	19.75	20.62	19.62	20.44	19.00	21.56	20.13	20.44
Diff.	−0.81	....	−0.44	....	−0.87	....	−0.82	....	−1.56	....	−0.31	....
Fat (Ether extract) %	23.79	15.86	20.30	12.67	13.44	12.14	19.47	13.60	18.72	11.35	15.60	14.57
Diff.	+7.93	....	+7.63	....	+1.30	....	+5.87	....	+7.37	....	+1.03	....
Fat-free dry matter %	20.45	23.56	21.81	22.57	23.77	23.25	21.39	23.93	22.52	24.45	22.85	23.60
Diff.	−3.11	....	−0.76	....	+0.48	....	−2.54	....	−0.93	....	−0.75	....
Ash %	3.87	3.83	3.44	2.53	3.57	3.32	3.76	3.46	3.75	3.21	3.89	3.41
Diff.	+0.04	....	+0.91	....	+0.25	....	+0.30	....	+0.54	....	+0.48	....
Final live wt., gm.	203	272	187	282	185	252	190	269	180	239	183	270
Diff.	−61	....	−95	....	−67	....	−79	....	−59	....	−87	....
Final fat-free wt., gm.	155	229	149	246	160	221	153	232	146	212	154	231
Diff.	−74	....	−97	....	−61	....	−79	....	−66	....	−77	....
Total fat, gm.	48	43	38	36	25	31	37	37	34	27	29	39
Diff.	+5	....	+2	....	+6	....	0	....	+7	....	−10	....

in a very rapidly growing rat, is used for maintenance. The data reported in this paper are in agreement with the results obtained by Macallum (1919). He found that the calorific cost of a unit gain in weight was greater for females than for males.

Since the data presented in Table 3 and illustrated in Figure 2 indicate that the efficiency of food utilization is more closely related to body surface than to body weight, it seems advisable to calculate the efficiency indices by using the mean surface area of the animal instead of using the mean body weight. The major reason for not so calculating the indices is that surface area is not an easy quantity to measure with accuracy. Surface area is a calculated value based on the use of predetermined constants that vary with age, degree of obesity, sex, and other factors. Furthermore, the calculated values for the surface area are functions of body weight. It is questionable, in our opinion, whether it is advisable to substitute in the determination of the efficiency quotient a calculated factor (surface area) subject to a considerably greater error of measurement than a factor (body weight) which can be obtained very accurately experimentally. This is especially true when the efficiency index is used to compare the efficiency of food utilization of a control with a group of experimental animals. It is this type of experiment in which the efficiency index method probably will be most useful.

Chemical Composition of Whole Male and Female Rats

Reports in the literature point to a difference between the chemical composition of the sexes of other species, especially in fat and dry matter content. Mitchell, Card, and Hamilton (1926) report chemical analyses of white Plymouth Rock chickens, and Mitchell and Hamilton (1927-28) report some preliminary chemical analyses of beef heifers and steers. In the data from both of these reports, the female shows a distinctly higher percentage of dry matter and of ether extract than the male. Therefore, the lower efficiency of the female rat might be due, in part at least, to differences in the composition of the gains in weight, the female gains being higher than the male in content of dry matter and particularly of energy. Animals of both sexes having different efficiency indices were killed and analyzed according to the procedure already described. The results of the chemical analyses are given in Table 4, which shows that the female rats at an approximate age of 10 weeks are composed of a higher percentage of dry matter, fat (ether extract), and ash, and a lower percentage of nitrogen and fat-free dry matter, while there is no difference as shown in the last line of this table in the total amount of fat contained in the carcasses

of the two sexes. To obtain a statistical measure of the significance of these differences in composition, the method proposed by "Student" (1917) has been used. The actual differences in composition between each pair of animals are given in Table 4. The pairs were obtained by arranging all the animals of each sex according to their efficiency indices. The percentages of the different constituents as obtained for the males were then subtracted from those obtained from the females. The only item appearing in Table 4 that shows no significant difference between the two sexes is that of total fat. The statistical constants for these analyses are given in Table 5. It is apparent from Table 5 that female rats on this type of ration (high protein, high fat) build up their fat deposits per unit of live weight more rapidly than do males. It is of interest to note that the composition in total fat of the two sexes is the same. It is impossible to say to what extent this condition is influenced by the composition of the ration. It is, however, demonstrated that rapidly growing female rats on the diet used are able to divert per unit of live weight a greater portion of their diet to the fat depots than are male rats of the same age if it be assumed that their initial composition was the same. Table 5 shows that the percentage of fat-free dry matter in male rats is significantly higher than in females. This fat-free dry matter, apparently, is largely protein, since the fat and ash of the female are higher than those of the male. Thus the data indicate that the lower efficiency of the female in comparison with the male is due in part to her higher percentage of fat with its concomitant higher energy content. Conversely, the higher content of protein, and, therefore, of vital tissue, is apparently more economically attained in the male of this species than in the female.

The coefficients of variation for the various items given in Table 4 are given in Table 5. The statistics show that female rats are more variable than males in all their constituents studied except ash. The greater variations in the female rat, as shown in Table 5, furnish evidence for the desirability of using the male animal for growth studies in preference to the female.

Tables 4 and 5 show no very definite relationships between the efficiency quotients and the chemical composition. The percentages of fat for the female group show some slight indication that the more efficient animals had increased more rapidly in fat than in non-adipose tissue. The females with efficiency indices of less than 2.00 appear to be somewhat higher in percentage of fat than the animals having efficiency quotients higher than 2.20. There is a large amount of fluctuation, however, for it will be observed that rat No. 102 (Table 4) with an efficiency quotient of 1.90 has only 18.34 per cent fat while animal No. 119, efficiency quotient of 2.36, had 18.74 per cent fat. The least

Table 5

Statistical Constants for Chemical Composition of Rats Shown in Table IV

	Mean		Standard deviation		Coefficient variation		Mean difference	Standard dev. of difference	Z value*	Probability value
	Female	Male	Female	Male	Female	Male	Females–Males			
Efficiency index.....	2.08 ± .05	1.24 ± .03	0.26 ± .035	1.14 ± .02	12.60 ± 1.7	10.91 ± 1.60	+0.84	0.14	6.00	<0.9999
Dry matter %.....	42.04 ± .42	37.50 ± .28	2.22 ± .29	1.43 ± .21	5.27 ± .70	3.82 ± .55	+4.64	2.36	1.97	< .9999
Nitrogen %	3.04 ± .03	3.28 ± .02	0.14 ± .02	0.08 ± .01	4.57 ± .63	2.37 ± .33	−0.24	0.14	1.76	< .9999
Protein %.....	18.97 ± .17	20.53 ± .09	0.84 ± .12	0.47 ± .07	4.44 ± .61	2.28 ± .31	−1.48	0.25	5.95	< .9999
Fat %	20.20 ± .56	14.11 ± .31	3.00 ± .40	1.58 ± .23	14.88 ± 2.0	11.20 ± 1.63	+5.78	2.96	1.95	< .9999
Fat-free dry matter %	21.77 ± .18	23.48 ± .09	0.99 ± .13	0.48 ± .07	4.53 ± .60	2.04 ± .29	−1.47	0.29	4.97	< .9999
Ash %	3.62 ± .04	3.25 ± .06	0.20 ± .03	0.31 ± .04	5.64 ± .78	9.46 ± 1.3	+0.37	0.28	1.31	.9994
Final live wt., gm. ..	202.4 ± 3.5	277.4 ± 3.9	17.36 ± 2.5	19.49 ± 2.7	8.57 ± 1.2	7.02 ± 1.0	−75.00	10.6	7.07	< .9999
Final fat-free wt., gm.	161.6 ± 2.4	238.8 ± 3.0	12.03 ± 1.7	14.9 ± 2.0	7.45 ± 1.0	6.25 ± .87	−77.17	10.42	7.41	< .9999
Total fat, gm.....	40.2 ± 1.7	38.7 ± 1.3	8.74 ± 1.2	6.55 ± .09	21.77 ± 3.0	16.93 ± 2.3	+1.75	9.89	0.18	0.7395

* Z Value $= \frac{\text{mean}}{\sigma}$

σ = standard deviation

When the Z value = 1.6, Probability = 0.9999.

efficient animal, No. 288, has only 15.60 per cent fat and an efficiency of 2.55. If it were not for the exceptions noted above, the data would suggest that the female rat was simply transferring food fat to body fat without any expensive loss of energy. Such a suggestion is not supported very well by the male group altho, generally speaking, the less efficient animals appear somewhat lower in fat.

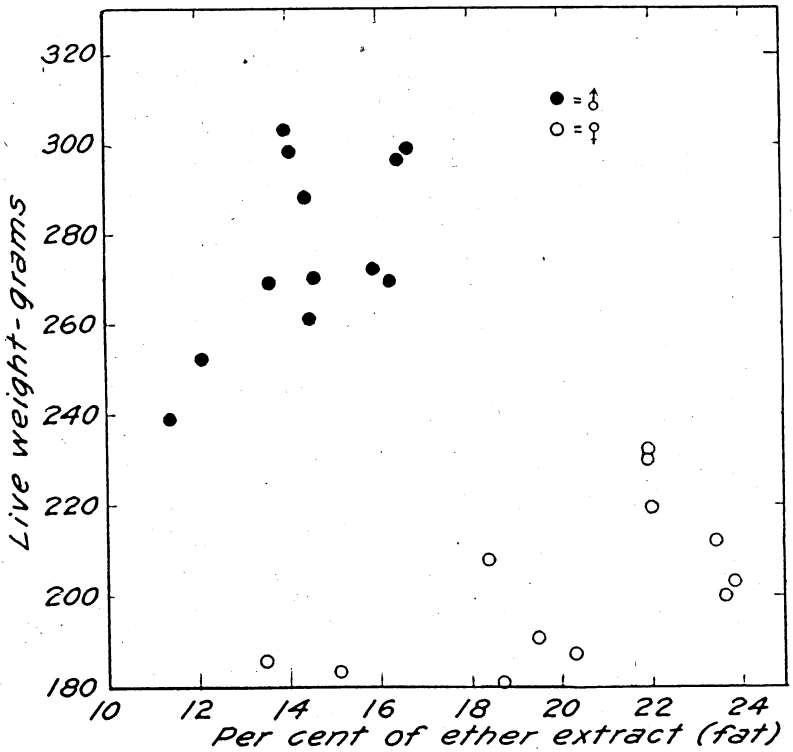


Fig. 3. Frequency diagram of the relation between the total gain and the percentage of ether extract of male and female rats fed the same diet.

Further study of this point has been made by plotting the live weight and fat-free live weight (ordinate) against percentage of ether extract (fat) and total ether extract (abscissa) in Figures 3 and 4 using the data presented in Table 4. Figure 3 shows a positive relationship between the body weight and the percentage of fat in both sexes. A less positive relationship is shown between the fat-free live weight and the total weight of ether extract in Figure 4. The latter illustration shows graphically what was pointed out above, that the

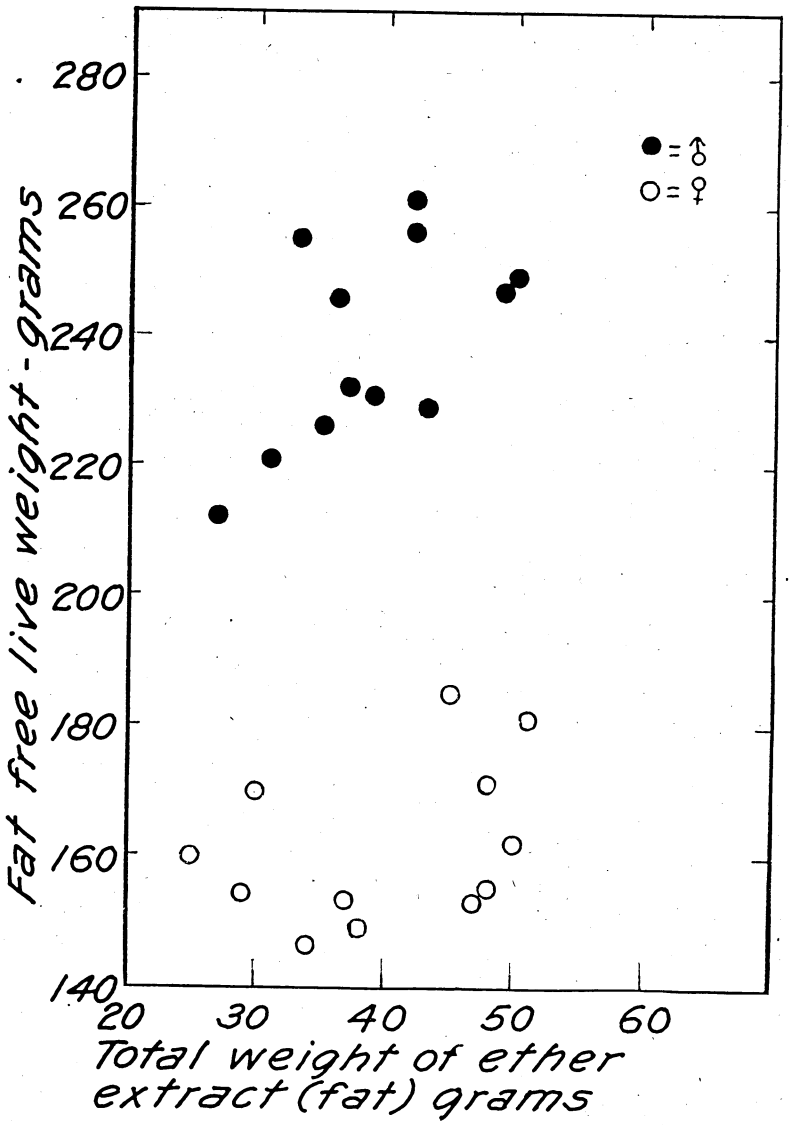


Fig. 4. Frequency diagram of the relation between the fat-free live weight and the weight of ether extract of male and female rats fed the same diet.

total amount of fat in the two sexes is about the same. It is noteworthy that there is a very definite separation of the two sexes as regards the fat percentages, a difference shown in Table 5 to be very significant statistically. These chemical analyses are insufficient to warrant general conclusions, altho they do raise the question as to what

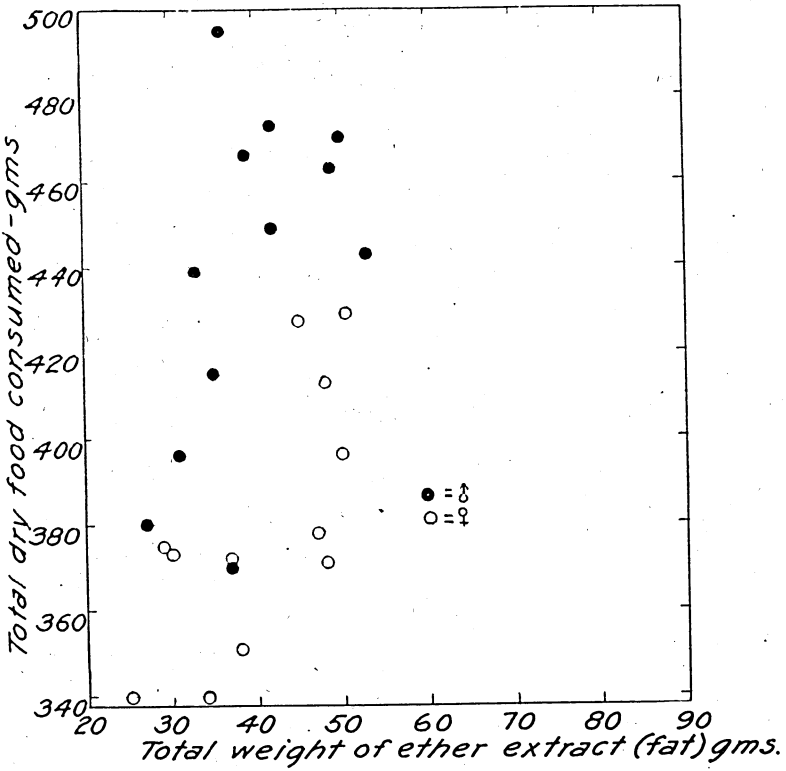


Fig. 5. Frequency diagram showing the relation between the food consumed and the amount of ether extract in the body of male and female rats fed the same diet.

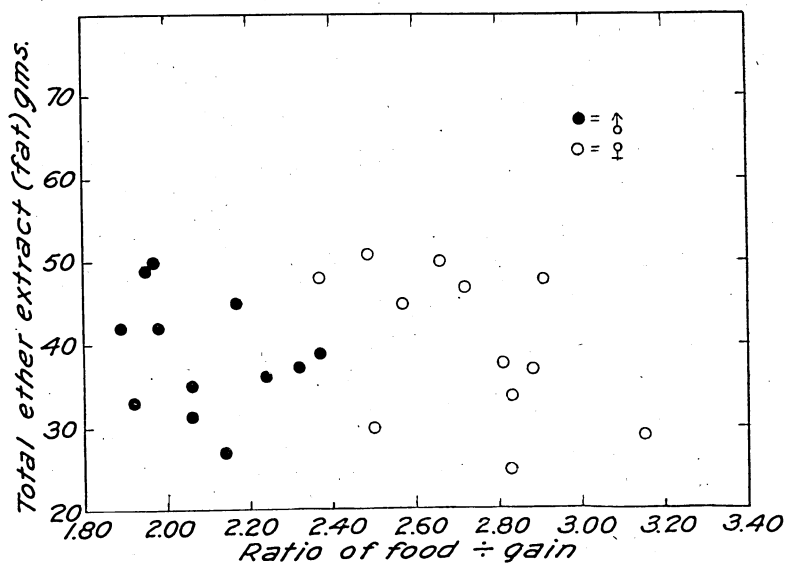


Fig. 6. Frequency diagram of the relation between the total ether extract in the body and the food required per unit of gain in male and female rats fed the same diet.

extent the apparently rapid growth attained by young rats upon the diet used in this experiment was due to an increase in body fat. The total amount of body fat is not related to the total food consumed, for Figure 5 shows that for male rats there appears to be no difference between the total body fat of the animals consuming the most food and those consuming the least food. Figure 5 also shows that for female rats there is only a very slight indication of a positive relation between the total dry food consumed and the total body fat (ether extract).

Figure 6 shows graphically the relation between the total fat (ether extract) and the ratio of total dry matter consumed per unit gain in live weight. A negative relation would indicate that the rats using less dry matter per unit gain in weight were taking advantage of the high fat content of the diet to form more adipose tissue; a positive relation would indicate that the adipose tissue deposited was formed at a greater food cost per unit gain, and, therefore, that the animal was probably synthesizing adipose tissue from the diet. Figure 6 indicates a very slight trend toward a negative relation for both groups. It may be inferred from this figure that part of the adipose tissue of both sexes was derived directly from the food fat.

Length of the Intestines as Related to the Efficiency Index, the Body Weight, and the Dry Matter Consumed

Even tho Palmer and Kennedy (1931) found little variation between different rats in the digestibility of the dry matter of the diet, it was thought that there might be some relation between the capacity of a rat to consume food and its efficiency. It would be exceedingly difficult, however, to make sufficiently accurate measurements of the volume of a rat's alimentary canal to determine its capacity. On the other hand, it was thought that some indication of the capacity of the rat's alimentary tract could be obtained by measuring the length of the small and large intestines and the caecum. These measurements have been made on 64 male and 53 female rats. The values for these measurements appear in Figures 7a, 7b, 8a, 8b, 9a, 9b, 10a, and 10b. With these measurements and with data on food consumption, live weight, and efficiency index, it has been possible to compare a number of correlation coefficients obtained for the two sexes. These correlations were made by combining the length measurement of the small and large intestines and the caecum into a value termed the "total length" of the intestinal tract. This value was correlated with the weight, with the dry matter consumed, and with the efficiency. In addition, a correlation was made between the food consumed and the live weight. Each of these correlation coefficients was calculated separately for each sex. Table 6

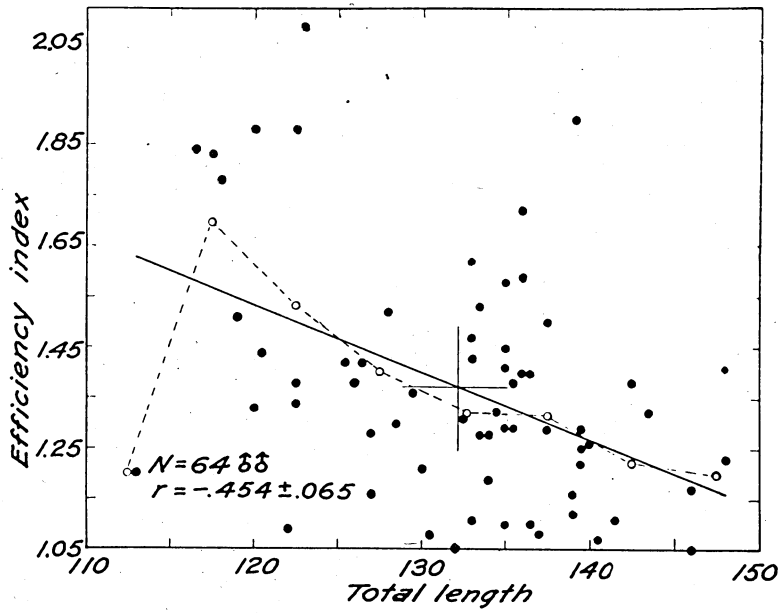


Fig. 7a. Frequency diagram of the relation between the efficiency index and the total length of intestinal tract in 64 male rats. Solid line is the regreession line. Broken line is the average efficiency index for different classes of intestinal length.

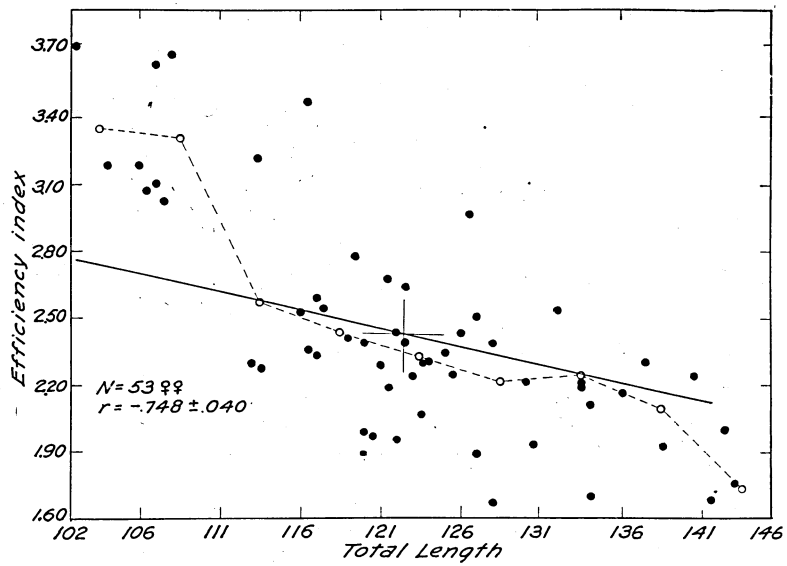


Fig. 7b. Same as Figure 7a for 53 female rats.

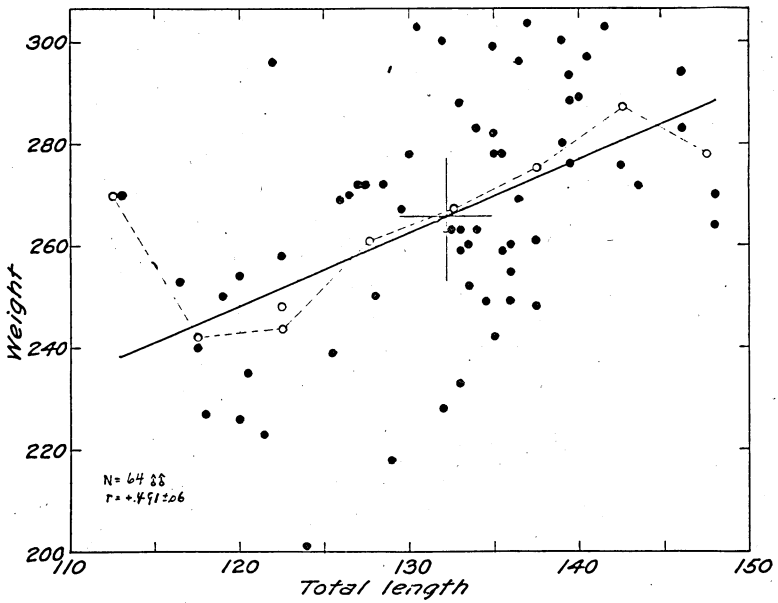


Fig. 8a. Frequency diagram of the relation between the live weight and total length of intestinal tract in 64 male rats. Solid line is the regression line. Broken line is the average live weight for different classes of intestinal length.

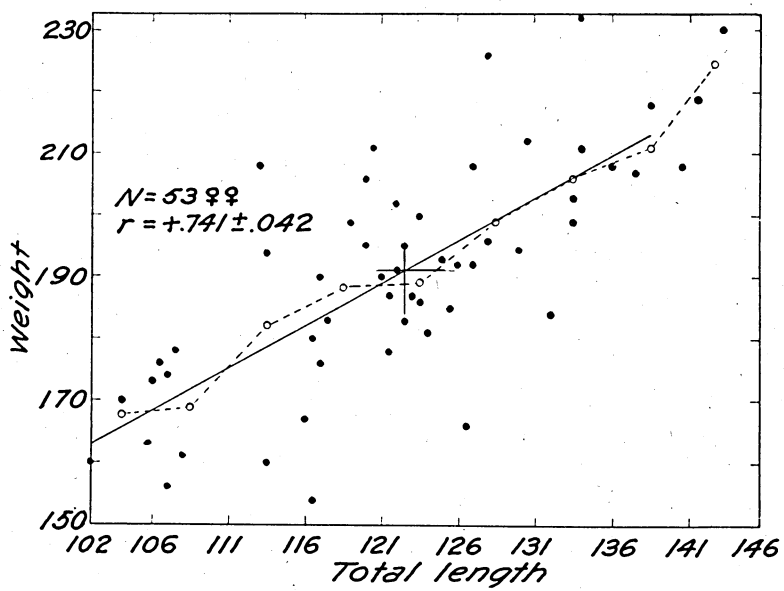


Fig. 8b. Same as Figure 8a for 53 female rats.

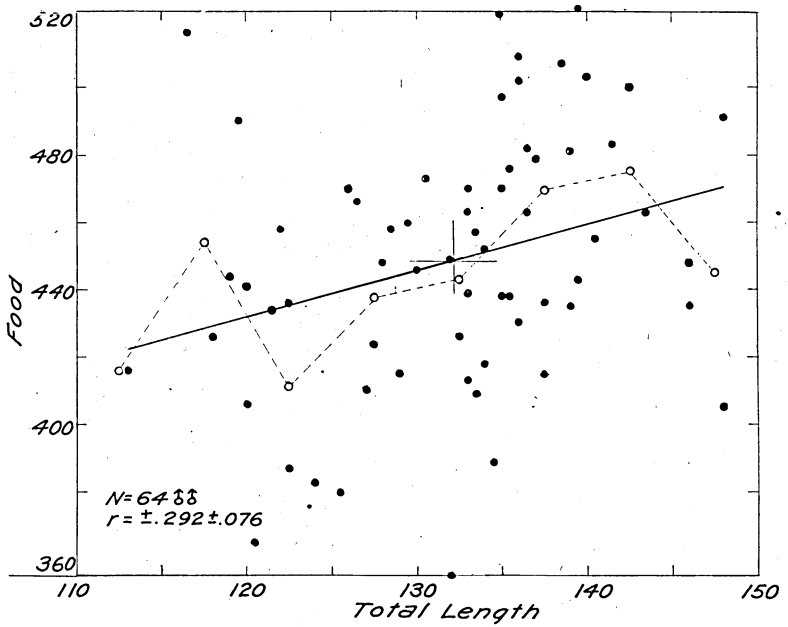


Fig. 9a. Frequency diagram showing relation between total dry matter consumed and total length of intestinal tract in 64 male rats after six weeks of linear growth. Solid line is the regression line. Broken line is the average consumption for different classes of intestinal length.

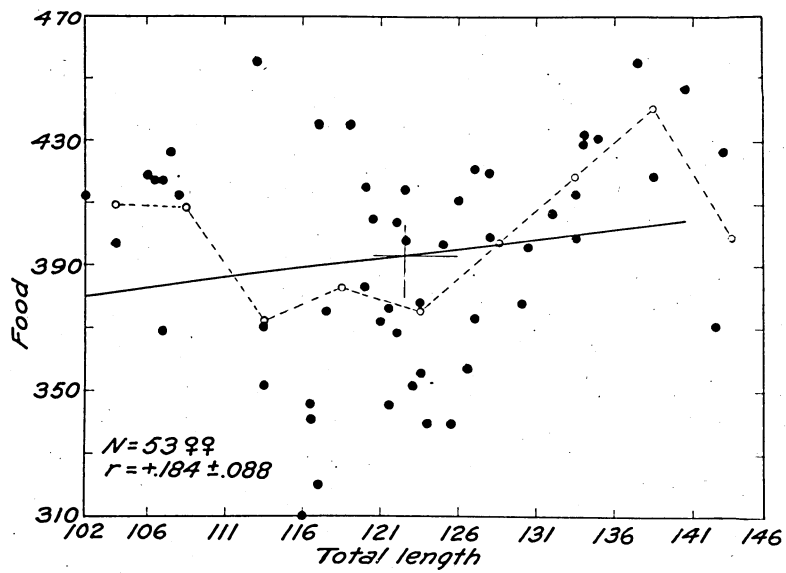


Fig. 9b. Same as Figure 9a for 53 female rats.

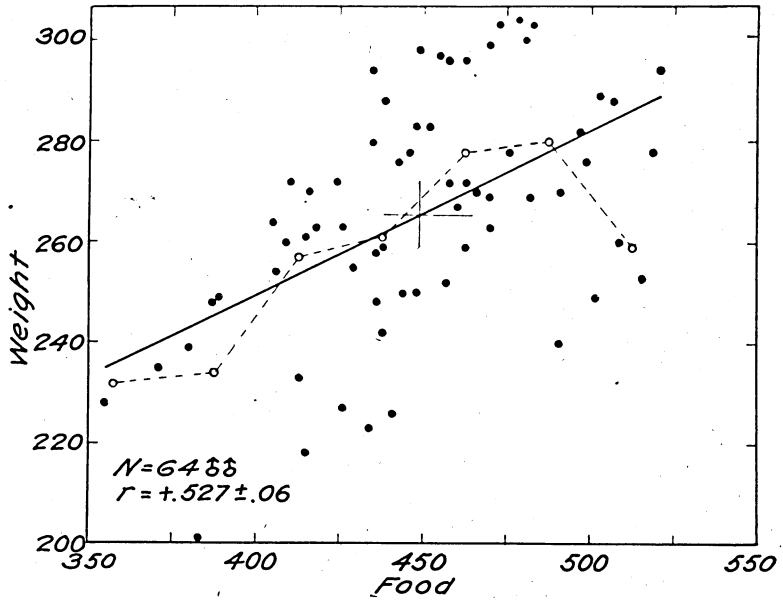


Fig. 10a. Frequency diagram of relation between total dry matter consumed and the live weight after six weeks of linear growth for 64 male rats. Solid line is the regression line. Broken line is the average live weight for classes of food consumption differing by 10 grams.

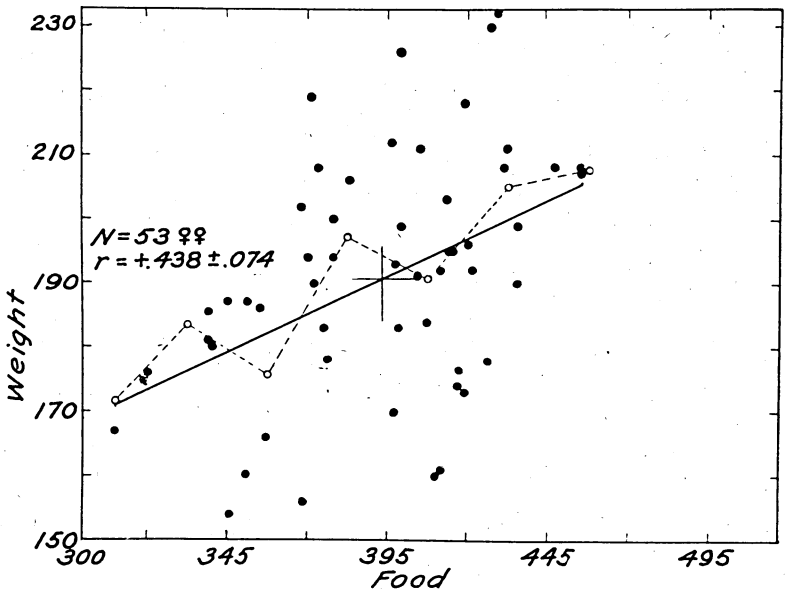


Fig. 10b. Same as Figure 10a for 53 female rats.

shows the numerical coefficients obtained, and Figures 7a, 7b, 8a, 8b, 9a, 9b, 10a, and 10b show the frequency diagrams for these correlations with the exception of the partial correlation, $r_{TW \cdot F}$, which is not illustrated.

Table 6

Correlation Coefficients as Calculated from Data on 64 Male and 53 Female Rats

E = Efficiency index	T = Total length of intestines
W = Live weight	F = Dry matter consumed
r = Correlation coefficient	

Males	Females
$r_{TE} = -0.454 \pm .065$	$r_{TE} = -0.748 \pm .040$
$r_{TW} = +.491 \pm .064$	$r_{TW} = +.741 \pm .042$
$r_{TF} = +.292 \pm .076$	$r_{TF} = +.184 \pm .088$
$r_{FW} = +.527 \pm .06$	$r_{FW} = +.438 \pm .074$
$^{*}r_{TW \cdot F} = +.519 \pm .063$	$^{*}r_{TW \cdot F} = +.747 \pm .040$

* A partial correlation where correction has been made for variation in F by use of the following equation:

$$r_{TW \cdot F} = \frac{r_{TW} - (r_{TF})\,(r_{WF})}{\sqrt{1 - r^2_{TF}}\ \sqrt{1 - r^2_{WF}}}$$

For both sexes there is a high negative correlation between total length of intestines and efficiency index. The females show a higher negative correlation than do the males. The negative correlations mean that, in general, the less efficient animals have a shorter intestinal tract.

The correlation for the males between total length of intestines and weight is $+ .491 \pm .06$ while a similar correlation for the females is $+ .748 \pm .04$. That the intestinal tract would increase in size as the animal grows larger is to be expected, but why this correlation coefficient for the males should be only two-thirds as high as for the females is difficult to understand. The correlation between the total length of intestines and the food consumed is positive, but is too low to be significant; no appreciable difference appears between the sexes. The correlation between food and weight is in the positive direction and is signficant, altho not so high as the correlation between the same variables for a larger population shown in the following section. The frequency surfaces for these correlations show considerable variation from the regression line altho the average ordinate values for different classes on the abscissa follow the regression lines as well as could be expected for the number of individuals included in the calculations.

The partial correlation coefficient between total length and weight, when a correction has been made for the variation in food consumption, shows only a very slight effect due to variations in food consumption. It appears, therefore, from this partial correlation coefficient and from the very low correlation between intestinal length and food consumed that, insofar as these measurements are concerned, the length of the intestines does not influence the food consumption of the rat to any appreciable extent. On the other hand, the definite negative correlation between the efficiency index and the total length of the intestines is probably due largely to the fact that the more efficient rats attain a greater body size during the test period. This explanation is indicated by the positive correlation between body weight and intestinal length.

Correlation Between Dry Matter Consumed and Gain in Live Weight That Indicates Certain Inadequacies of the Paired-Feeding Method

It will be recalled that there was a fairly high correlation between food consumed and live weight, as shown in Figure 10. Since there were only 53 female and 64 male animals included in the correlations, a further statistical study of the relationship between the total food consumption and the gain in live weight was made on a much larger group of animals.

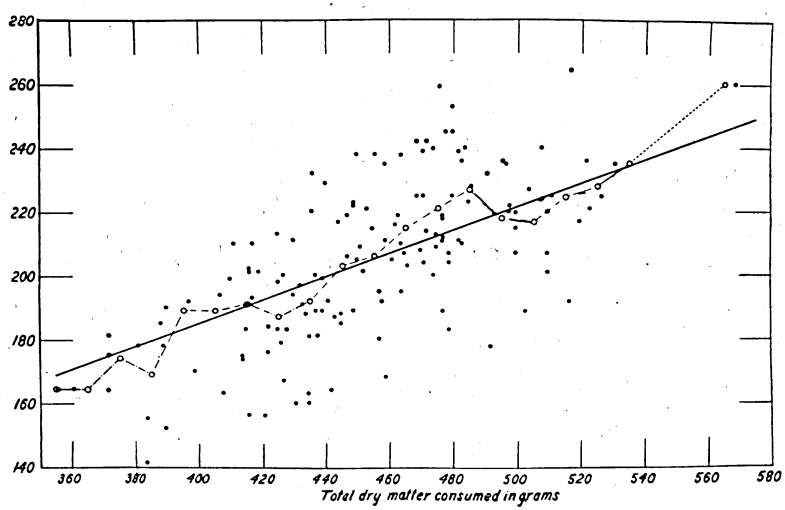


Fig. 11. Frequency diagram of relation between gain in weight and dry matter consumed after six weeks linear growth on same diet for 146 male rats. Solid line is the regression line. Broken line is the average gain for the classes of food consumed (differing by 10 grams).

Figure 11 shows the variables on a frequency surface for 146 male rats over a six-week experimental period. This frequency surface represents a correlation coefficient of + .676 ± .030. The correlation surface for 141 females (Fig. 12) is very similar to the one shown for males, altho the swarm is somewhat shorter and the correlation coefficient + .642 ± 0.33, somewhat lower.[6]

One of the most important factors, which interferes with a measure of the quantitative differences between two diets, is that of giving the correct interpretation to the food consumption. The records of gain in live weight and of dry food consumed for these two groups of rats make available data which lend themselves to statistical analysis and can be used to test the validity of certain fundamental assumptions in the use of the "paired-feeding" method advocated by Mitchell and Beadles (1930). By this method the above workers propose to eliminate the difficult problem of interpreting the food consumption. In

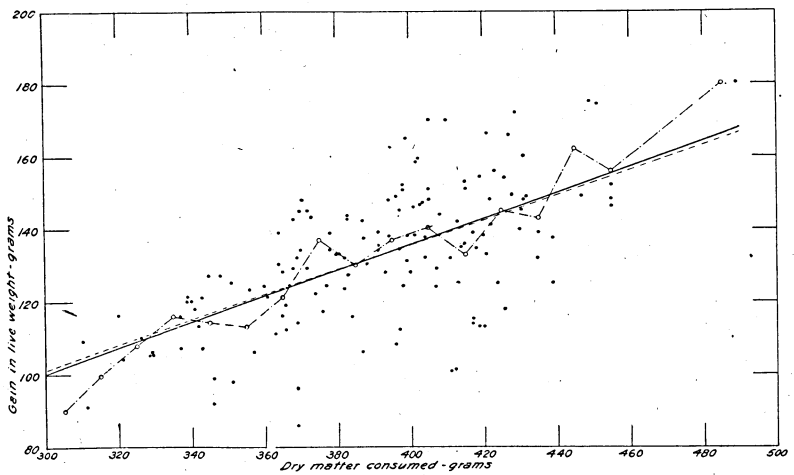


Fig. 12. Same as Figure 11 for 141 female rats.

their procedure the animals are divided into pairs of equal weight at the beginning of an experiment. The food intake of each pair is strictly equalized throughout the experimental period, and the difference in the gain of the two animals is ascribed solely to the difference in the two rations under investigation, which differ by only one variable.

[6] It is quite remarkable that Mrs. Irwin (1932), in a recently published calculation of the correlation between the total gain and the mean daily food intake of 123 standard Wistar strain rats during a 36-39 day depletion period on vitamin A-free diet, gave a value of r = .64 ± .04. Both sexes were evidently included in Mrs. Irwin's study. It appears, therefore, that a typical random sample of any inbred population of albino or hooded rats will show essentially the same correlation between food consumption and gain in weight regardless of the type of diet, provided, of course, that gain in weight is continuous and "normal" for the particular diets studied.

In the use of this method in the interpretation of dietary differences, the assumption is made that the two animals would make the same use of their food for growth if it were of the same composition, i.e., there would be a perfect correlation between the food consumed and the gain in live weight. By such an assumption it is also admitted that there are slight, or no, individual differences in the ability of animals to gain in live weight when they consume the same quantity of the same dietary, i.e., each animal of a pair should make a corresponding increment of gain for every increment of food consumed, and if they do not, when the diets differ by one variable the difference in the gain is a measure of the difference in the two diets. The data shown in Figures 11 and 12 illustrate the wide variation in gain in live weight which may be obtained on equal food consumption during the period when growth is taking place at the most rapid rate, a period in which growth is essentially a linear function of time and in which we would expect, therefore, to find the highest correlation between food consumed and gain in live weight.

The correlation coefficients calculated for two large groups of male and female rats, $+ .676 \pm .03$ and $+ .642 \pm .033$, respectively, show that there is not a perfect correlation between food consumed and gain in live weight and, therefore, that the equalization of the food consumption between pair mates does not give sufficient control over a growth experiment to justify, as indicated by Mitchell, a "clear interpretation of the results with reference to the one deliberately imposed variable." The correlation between gain in live weight and dry food consumed is only 65 per cent, and there are also wide variations in growth of rats when they consume the same quantity of the same food over a six weeks' experimental period. The data in Figures 11 and 12 show that rats having the same food consumption make widely different gains in live weight. To cite just one specific example by way of illustration—our records show that male rat No. 78 gained 212 grams in live weight on 476 grams of dry matter while male rat No. 60 gained 259 grams in live weight on the same food consumption, a difference of 47 grams in 6 weeks. This pair of rats was on trial under the same conditions as to age, length of period, time, etc., and so it does not seem justifiable to ascribe this wide variation in growth to environmental differences, since the similar environment should affect both animals alike. To cite another example, rat No. 48, Table 5, gained 229 grams on 439 grams of food while rat No. 45 gained 201 grams on 415 grams of food; it is obviously impossible for the rat consuming 24 more grams of dry matter to gain 28 more grams in weight if the efficiency of food utilization is the same. This second pair of animals is included in the data

given in Figure 11. Yet from the chemical analyses (Table 5) it will be seen that the rat making the lesser gain in live weight contained 35 grams of fat while the animal making the greater gain in live weight contained 33 grams of fat at the end of the experiment. It is believed that the chemical analyses are not sufficient to explain the difference in gain. The examples cited are not the most extreme cases, neither are they the most uniform ones.

The data presented in Figures 11 and 12 show that animals on the same diet making the same gain require widely differing amounts of food. If such animals were paired and the amount of food determined that was used to make these equivalent gains, there would be as wide fluctuations as were found when pairs of rats ate the same amount of food but made widely differing gains. This observation, since the diet of the animals was constant, offers a measure of the variability of the animals and does not indicate a true measure of differences in two differing diets. In discussing the paired-feeding method, Mitchell (1930) says, "There are many reasons for believing that the ideal paired-feeding experiment would involve not the determination of the gains resulting in paired animals from the consumption of equal amounts of the rations being compared, but rather with the determination of the amounts of the rations necessary to promote equal gains in paired animals. The former plan is the more feasible, but the latter theoretically the more perfect." We have just pointed out in Figures 11 and 12, however, that the experimental determination of the amounts of the same ration necessary to give equal gains is not more perfect. If it were possible to pair animals of the same efficiency of food utilization and keep this efficiency for the pair mates the same throughout an experiment, then it would be possible to determine with a great deal more accuracy the amount of food required to produce equal gains. Then and only then can it be said that the paired-feeding method would adequately interpret the differences between two dietaries that differ by only one variable. Such an ideal may never be attained, as it is doubtful if it will ever be possible to obtain animals all of which have a uniform efficiency of food utilization that will remain uniform over a long experimental period. It is obvious that the larger the number of pairs of animals included in this type of experiment the less would be the effect of these individual variations on the interpretation of the results of the experiment.

An interesting comparison has been made between the differences in gain in live weight of 9 pairs of male rats of the same age consuming voluntarily the same amount of the same diet during a period of 6 weeks and the differences in gain of the same number of pairs when

the food consumption was experimentally controlled and the diet was made variable. For this comparison 9 pairs of rats shown on Figure 11 were used. The essential data for this comparison are given in Table 7. The differences in gain of this group of animals are compared with data shown in Table 8, which are taken from the published results of one of Mitchell and Beadles (1930) experiments. The data chosen for this comparison are those in which the length of the experimental period was most comparable to the period used in this investigation. By comparing the data in these two tables it will be observed that there were greater differences in the gain between two animals consuming voluntarily the same quantity of the same diet than was obtained by Mitchell and Beadles in diets differing in one constituent. The animals in Table 7 were especially selected to show maximum variations that may be obtained between animals. Such facts, however, further indicate that consideration should be given not only to the food consumption but also to the efficiency of food utilization before ascribing differences in the gain solely to the differences in the two diets.

Correlation Between Dry Matter Consumed and Deviation of the Actual Gain from Its Most Probable Value

Having the correlation coefficient between food and gain, it is possible to calculate the correlation coefficient between the food consumed and the deviation from the most probable gain by means of a formula derived by Harris (1909) in which

$$r_{FZ} = \frac{r_{FG} - V_G/V_F}{\sqrt{(1 - r^2_{FG}) + (r_{FG} - V_G/V_F)^2}}$$

in which F = food consumed (the independent variable)

Z = G − PF, (P = $\overline{G}/\overline{F}$) or the deviation of the gain of any individual from its most probable value, a dependent variable.

V_G = the coefficient of variation for the gain.

V_F = the coefficient of variation for the food.

and r = the correlation coefficient.

Clearly, the most probable gain for any individual is PF, in which P equals the ratio of the average gain ($\overline{G}$) to the average food ($\overline{F}$) for the whole population studied. We may take Z = G − PF, or the deviation of the actual gain of an individual from its most probable value. To obtain a line representing the most probable gain, it is only necessary to obtain the product of PF. This PF line is illustrated as a straight dotted line in Figure 12, the correlation surface showing the relation between total dry matter consumed and gain in live weight

Table 7

Data for Nine Pairs of Male Rats Shown in Figure 11 Selected as Examples of Natural Paired Feeding

Pair	Initial weight	Final weight	Gain	Total dry feed	Difference in gain
	gm.	gm.	gm.	gm.	gm.
1	61	253	192	516	
	60	324	264	516	72
2	65	248	183	478	
	62	315	253	479	70
3	64	259	195	463	
	58	296	238	463	43
4	60	252	192	457	
	61	296	235	458	43
5	64	244	180	456	
	59	297	238	455	58
6	62	226	164	441	
	59	288	229	439	68
7	59	219	166	434	
	62	294	233	435	70
8	58	218	160	430	
	59	270	211	429	51
9	64	216	152	389	
	59	249	190	389	38

Table 8

Value of Cystine as a Supplement to the Proteins of Whole Milk

Length of Feeding Period 70 days. Data from Mitchell and Beadles (1930)

Pair	Feed	Initial weight	Final weight	Gain	Total food	Difference in gain
		gm.	gm.	gm.	gm.	gm.
1	Control	57	163	106	717	
	Cystine	56	177	121	712	15
2	Control	55	153	98	599	
	Cystine	52	152	100	606	2
3	Control	51	146	95	662	
	Cystine	51	164	113	657	18
4	Control	47	141	94	613	
	Cystine	45	165	120	611	26
5	Control	40	152	112	619	
	Cystine	40	189	149	662	37
6	Control	46	148	108	594	
	Cystine	41	173	132	594	24
7	Control	41	135	94	595	
	Cystine	37	171	134	595	40
8	Control	32	128	96	575	
	Cystine	34	164	130	178	34
9	Control	34	137	103	544	
	Cystine	34	151	117	541	14

for a group of 141 female rats. The r_{FZ} correlation for this group was found to be + .004 ± .058, an insignificant value. Inspection of Figure 12 will show the slight deviations of the most probable gains of these animals from the actual gains as illustrated by the regression line. The correlation coefficient as determined by the above formula on 146 male rats was found to be — .005 ± .058. Since both these correlation values are very low and insignificant, we may conclude that the rats making greater gains eat more food actually but no more relatively than do those making lesser gains. In other words, the rats that consume very large quantities of dry matter are actually making the same use of their food insofar as the gains they make are concerned as are the rats consuming lesser amounts of food.

Correlation Coefficient Between Dry Matter Consumed and Gain in Weight with Increase in Length of the Experimental Period

It was thought that there might be a gradual increase in the correlation coefficient between gain in weight and dry matter consumed

Table 9

Correlation Between Gain in Weight and Total Dry Matter Consumed for 146 Male Rats for Experimental Periods of 1 to 6 Weeks

The longer periods include the shorter ones.

Length of period	Mean gain	Standard deviation of gain	Mean food	Standard deviation of food	r*
weeks	gm.		gm.		
1.....	35.43 ± .34	6.12 ± .24	55.08 ± .32	5.65 ± .22	+0.396 ± .047
2.....	72.56 ± .60	10.82 ± .43	123.27 ± .63	11.33 ± .45	+ .548 ± .037
3.....	108.91 ± .86	15.33 ± .61	200.28 ± 1.04	18.68 ± .73	+ .640 ± .032
4.....	146.31 ± 1.12	20.15 ± .80	282.02 ± 1.45	26.03 ± 1.03	+ .666 ± .031
5.....	178.03 ± 1.27	22.83 ± .90	366.50 ± 1.91	34.16 ± 1.35	+ .631 ± .035
6.....	204.52 ± 1.40	25.03 ± .99	452.22 ± 2.19	39.18 ± 1.55	+ .676 ± .030

* r = correlation coefficient.

Table 10

Correlation Between Gain in Weight and Total Dry Matter Consumed for 141 Female Rats for Experimental Periods of 1 to 6 Weeks

Cumulative periods.

Length of period	Mean gain	Standard deviation of gain	Mean food	Standard deviation of food	r*
weeks	gm.		gm.		
1.....	30.66 ± .30	5.32 ± .21	51.94 ± .30	5.23 ± .21	+0.458 ± .045
2.....	57.94 ± .54	9.53 ± .38	114.60 ± .63	11.02 ± .44	+ .524 ± .041
3.....	80.30 ± .70	12.31 ± .49	181.84 ± .81	14.30 ± .57	+ .579 ± .038
4.....	100.67 ± .87	15.29 ± .61	250.52 ± 1.31	23.03 ± .93	+ .568 ± .038
5.....	117.84 ± 1.01	17.73 ± .71	318.10 ± 1.72	30.33 ± 1.22	+ .578 ± .038
6.....	131.37 ± 1.31	23.00 ± .92	388.83 ± 2.02	35.53 ± 1.43	+ .642 ± .034

* r = correlation coefficient.

the longer the animals were on the experiment. Hence, the correlation coefficients for the group of 146 male and 141 female rats have been calculated for periods of 1, 2, 3, 4, 5, and 6 weeks. Tables 9 and 10 and Figure 13 show the results of these calculations. For both males and females, after a rapid increase from the first to the third week, the correlation coefficient remains practically constant, with a slight, though insignificant, rise for the period six weeks in length.

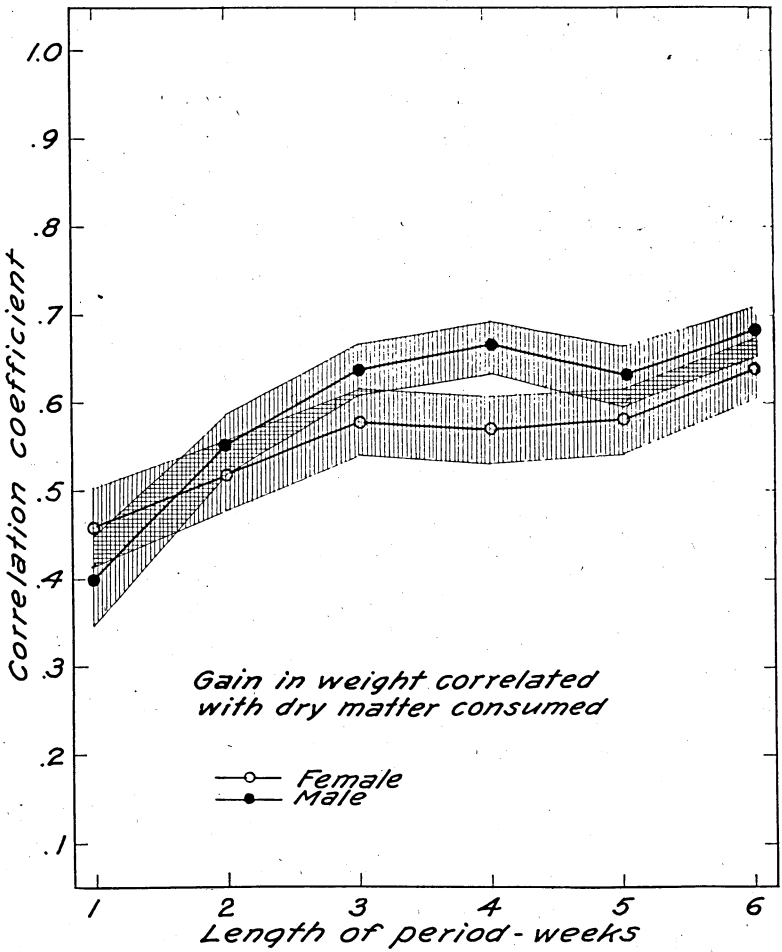


Fig. 13. Correlation coefficients between gain in weight and dry matter consumed for 146 male and 141 female rats for periods of one to six weeks. Shaded areas show the probable error of the means.

Effect of Selection Upon the Correlation Between Gain in Live Weight and Amount of Dry Food Consumed for an Experimental Period of Six Weeks

As these investigations were originally attempted to increase the uniformity of the rat as an experimental animal for growth experiments, it would be highly desirable to know what effect selection of animals for high efficiency has had upon the correlation between gain in live weight and amount of dry matter consumed. Sixty-five male rats, all those available, in the fourth to eighth generations, inclusive, have been used in this study. The correlation coefficient was found to be $+ .57 \pm .055$, essentially the same as a similar correlation which was found for 146 male rats consisting largely of an F_2 population.

When consideration is given to the statistical constants for the two groups of animals, it will be observed in Table 11 that the average gain in weight for 65 males was 209.0 ± 1.38 gm. whereas for the group of 146 males, the average gain was 204.5 ± 1.40 gm. and the amount of dry food consumed was found to be 444.0 ± 2.4 gm. and 452.2 ± 2.19 gm., respectively, for the two groups. While these differences are of questionable significance statistically for the numbers employed, the variability of both the gain and the food consumed is less for the group of 65 than for the one of 146 males. This is shown by the following coefficients of variation: C. V. for gain by 65 males, 7.8; C.V. for gain by 146 males, 12.2; C.V. for food consumed by 65 males, 6.6; C.V. for food consumed by 146 males, 8.7. If Figure 14, the frequency surface for the 65 male rats, is compared with the frequency surface for the larger group of males, Figure 11, it at once becomes apparent that considerable progress has been made in decreasing the variability of the smaller group.

Table 11

Comparison of the Statistical Constants of Gain and Food Consumed for 65 Selected and 146 Unselected Male Rats

	65 male rats 4th to 8th generations	146 male rats F_2 population
Average gain, gm.	209.0 ± 1.38	204.5 ± 1.40
Average dry matter consumed, gm.	444.0 ± 2.4	452.2 ± 2.19
Standard deviation of gain	$16.4 \pm .97$	$25.0 \pm .99$
Standard deviation of food	29.3 ± 1.73	39.2 ± 1.55
Correlation coefficient	$+0.57 \pm .055$	$+0.676 \pm .030$

The standard deviation for the gain of the 65 male rats is $16.4 \pm .97$, while for the 146 males it is $25.03 \pm .99$. The standard deviation for the food consumed is 29.3 ± 1.73 for the selected group; for the

other group it is 39.18 ± 1.55. These figures indicate that further progress by selection and inbreeding may be expected toward securing greater uniformity in our experimental animals. It is believed that the difference in the correlation coefficients in the two groups can be explained by the greater length of the swarm in the large group because it is known that an increase in the length of a correlation surface increases the correlation coefficient.

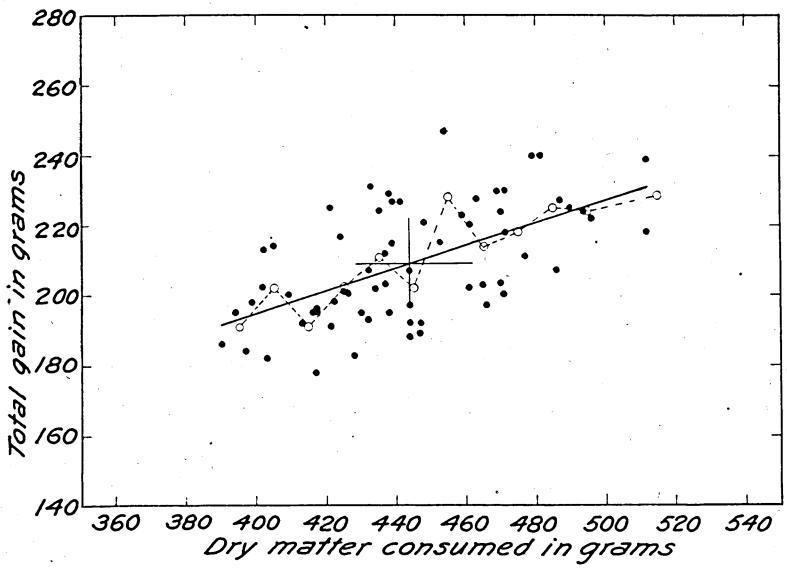


Fig. 14. Frequency diagram showing relation between total gain in live weight and dry matter consumed for 65 male rats in the 4th to 8th generations after six weeks linear growth on the same diet.

Effect of Feeding Dry Supplements Upon the Efficiency Index

The diet used for these investigations contained fresh head lettuce and fresh beef liver supplements. Since the lettuce came from several sources throughout the different seasons of the year, it was decided to test the effect upon efficiency after both supplements had been dehydrated at low temperature and pressure. It was believed that, providing the drying had no deleterious effect on the growth promoting qualities of these supplements, a more uniform and satisfactory diet could be obtained for further study of efficiency. Twenty-one rats from three different litters, two of the same mating, were tested for efficiency in the usual way except that the supplement in the diet was given in the dehydrated form. The results indicate thus far that dehydration of the liver and lettuce has no effect on the efficiency index. The average efficiency of the 12 male rats was 1.27 compared to an average of

1.38 for a large group of males receiving the fresh supplement. For the 9 females the average efficiency index was 2.27 compared to an efficiency index of 2.36 for females receiving the fresh supplements. There was no effect on the digestibility of the diet containing the dried supplements. In two weekly periods for 6 rats the apparent coefficient of digestibility averaged 92.79 and 92.81 per cent with a range of 92.01 to 93.49. This digestibility coefficient is essentially the same as that obtained by Palmer and Kennedy (1931) on rats receiving a similar diet containing fresh supplements.

Comparison of the Efficiency Index for a Period of Three Weeks with That for the Second Three Weeks of a Six Weeks Experimental Period

In order to study the possibility of using the determined efficiency of food utilization of an animal in a preliminary trial as an index of its efficiency for an experimental period that immediately followed, correlations have been made between the efficiency index of the first three-week period and that of the second three-week period for 146 male and 131 female rats. The frequency surfaces for these two correlations are shown in Figure 15. The correlation between the efficiency indices for these two periods is greater for the male than for the female rats. Figure 15 shows a larger angle to the regression line in the male group but a higher correlation coefficient than for the female group. This is explained by the fact that the ratio of the standard deviations of the ordinate ÷ abscissa is much greater for the female than for the male group. Even tho these two groups are plotted on the same scale, the male rats show a greater correlation between the efficiency index of the first three weeks and that of the second three weeks than is found in the female group. From this comparison it becomes apparent that for growth studies the males are considerably better than the females. The efficiency of the animal in a fore period correlated with the efficiency in a succeeding period is greater for the male than for the female. Since it was shown in Figure 13 that the male rat has a greater variability in his efficiency index the first week than at any other time in a six weeks experimental period, it was thought that a still higher correlation coefficient might be obtained by correlating the efficiency indices for the second- and the third-week periods with the fourth-, fifth-, and sixth-week periods. This was calculated but the same correlation ($r = + .73$) was obtained as was obtained for the first three-week period correlated with the second three-week period. Further experimental studies on this subject, in which other factors are varied, are now in progress.

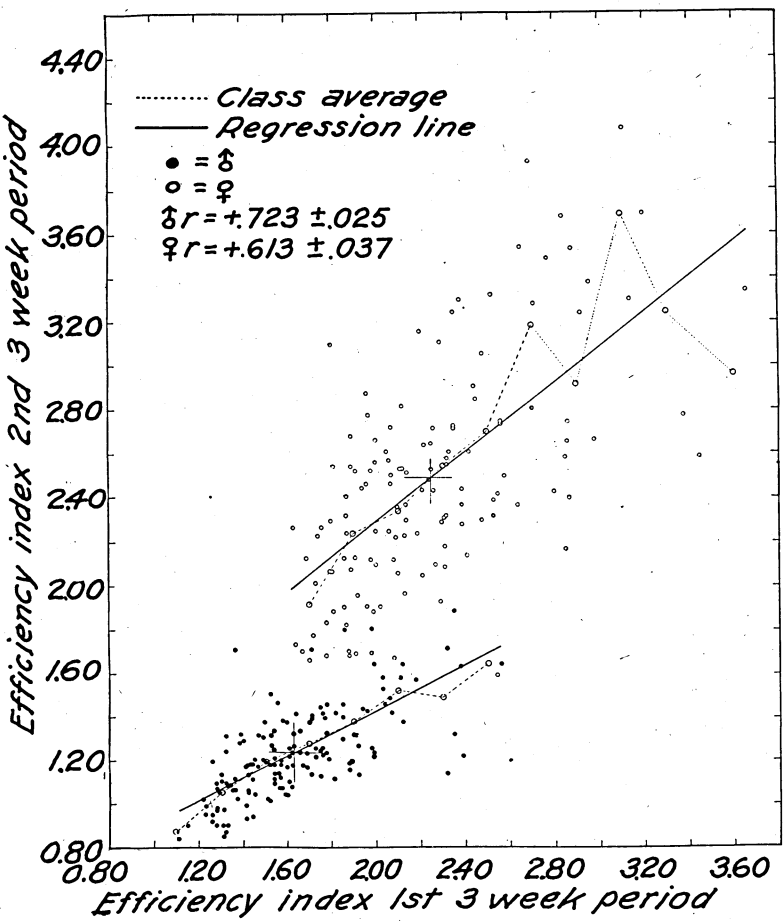


Fig. 15. Frequency diagram of the relation between the efficiency index of the second three-weeks period and that of the first three-weeks period for 146 male rats (lower diagram) and 131 female rats (upper diagram).

DISCUSSION

It can be assumed with a fair degree of safety that the ration used in this investigation provided the maximum dietary stimuli for growth, for in no case does an examination of the literature reveal feeding experiments in which the growth rate of rats exceeds that attained by the animals reported in this experiment. While that fact does not, of itself, prove that optimum growth has been provided, it does give good indication that insofar as our present knowledge of optimum nutrition for growth is concerned, the diet fed the experimental animals stimulated growth at the maximum rate. With uniform laboratory tem-

perature and with uniform care of the animals, it is believed that each rat was provided with conditions that created for it a uniformly constant and optimum environment. The amount of food consumed and the gain in weight resulting therefrom can be employed, therefore, in calculating an index that represents a measure of the efficiency of food utilization.

After an examination of the data it is apparent that we are dealing with highly variable material. The data presented in Figure 1 indicate that this variability is due, at least to some extent, to heritable factors. If the wide variability of the efficiency of food utilization obtained in the F_2 population and illustrated in Figure 1 be assumed to represent Mendelian segregation, this can not be explained on the basis of simple Mendelian factors. It seems probable that the F_2 segregation found was due to inheritable factors. The extent to which such variability of food utilization is determined by inheritance and the factors involved in its determination can not be studied until the animals have been inbred long enough to become homozygous. Figure 1 shows some of the variations observed in the first generations of various sib matings of pairs selected because of the likeness of efficiency index of the individuals in the pair. The data shown are not a complete report of this phase of the investigation, which is to be continued until the animals have become sufficiently homozygous to warrant a closer study of the inheritable factors concerned with food utilization.

The efficiency indices used in this investigation were calculated from the amount of dry matter consumed per unit of gain per unit of body weight. This is a logical method of procedure because it gives due consideration to an increase in maintenance cost. At the same time this method is less subject to errors of measurement than a method in which the efficiency index is calculated on the basis of a unit of body surface. With methods now in use, to measure accurately the surface area of a live rat is an impossibility. In experiments in which the experimental animal is to be used later for breeding purposes, it would be impossible to slaughter it in order to measure its surface area for an efficiency calculation. On the other hand, the surface area can be calculated, but any such calculation is, at best, subject to more than 3.5% error (Lee and Clark 1929). The accuracy of the formula actually used in this study for calculating surface area is questionable, also, because it was obtained by Lee and Clark on rats much older for the same weight range than those used in this study. The disadvantage of calculating surface area, however, when the formula is derived from measurements on totally unrelated animals in another laboratory, is not sufficient to invalidate the use of surface area in the calculation of an

efficiency index. Even with the possibility of introducing errors into the final efficiency index when calculated per unit of surface area, Table 5 shows a closer agreement between the ratio of dry matter consumed per unit of gain and the efficiency index expressed as a unit of surface area than is shown between the same ratio and the efficiency index expressed per unit of body weight. As previously pointed out, this is undoubtedly due to the fact that the maintenance cost is numerically the largest item involved in the cost of gain in weight.

In previous sections it has been noted that there are wide variations in food utilization in rats as indicated by the efficiency index method. The results also show that rats exhibit large variations in gain on the same food intake. A study of experiments on swine indicates that the rat is not unique in showing wide variations in the gains made on similar food intakes. Crampton (1928) has reported some data on growing pigs that were fed individually on the same diet. He observed wide fluctuations in the gains made on approximately the same food intake, e.g., one pig consumed 224 pounds of food and made a gain of 43 pounds. Another pig consumed 234 pounds of food and made a gain of 62 pounds; and still another pair consumed 283 and 290 pounds of food for gains of 52 and 73 pounds, respectively. While only ten hogs were studied in the feeding trials reported by Crampton, there is sufficient evidence to show clearly that swine may exhibit as wide individual variations in the efficiency of food utilization as those reported in this paper for rats. While it would not be justifiable to draw definite conclusions from the data of Crampton, his finding is highly suggestive of the applicability of the data secured in the present investigation on rats to problems of practical animal husbandry. The data secured in these trials undoubtedly include a sufficiently large number of individuals to make the conclusions drawn from them of biological significance and the results probably of considerable application to the larger domestic animals.

The study of the food consumption as related to the gain in live weight of nearly three hundred rats has shown that there is a comparatively low correlation between these two factors. At the same time it has also been shown that greater variations in gain in weight may be obtained on the same "super" diet than other investigators have found for paired animals on diets differing in only one constituent. It becomes obvious from such studies that "paired feeding" is not fully adequate to give a measure of the quantitative difference between such diets. While it is undoubtely true that controlled food intake of paired animals marks a progressive step toward the solving of the complicated problem of properly interpreting the effect of food intake upon the

growth of the animal, this method alone will not wholly suffice to permit a clear interpretation of food consumption data.

At the present time two other additional means appear to lend themselves favorably toward a clearer interpretation of this problem with rats. It is believed, in the first place, that only male rats should be used for growth studies because they are less variable in both their growth and food consumption and because they show a higher correlation between gain in live weight and food consumption. To limit experiments to male animals only, however, would necessitate the use of a considerably larger number of animals in the breeding colony and would not be adapted to experiments in which studies of reproduction and lactation are to be made. In the second place, it is believed that a further refinement of the methods of interpretating growth studies may be obtained by placing all the animals on the same diet for a short preliminary period of from two to three weeks immediately following weaning time. A longer preliminary period would be desirable, but such a period would, it is believed, be too great a sacrifice of the growth impulse which shows the greatest impetus during the first few weeks after weaning. At the end of this preliminary period the animals should be divided by pairs such that pair mates would have the same efficiency of food utilization. One of these pairs may then be placed on the control diet; the other one is placed on the experimental diet. The data from such paired animals lends itself admirably to statistical treatment by "Students" (1917) paired method. This particular mode of analysis has been used by several investigators in the field of animal nutrition and its use and application to nutrition problems has been adequately described by Crampton (1931).

A third possibility for further refining the methods of interpreting food intake is indicated by the studies reported herein. By methods of breeding and selection, families of rats can be produced that differ widely in their efficiency of food utilization for growth. This fact suggests that some progress can be made toward increasing the uniformity of our laboratory animals for experimental purposes. This is an attractive line of investigation to follow but it would require years of careful breeding and selection under strictly controlled environmental conditions before one could hope for any great amount of success. Four years of such work at this station have shown slight positive progress along this line. It was indicated in comparing Figures 11 and 14 that the variation in both growth and food consumption was less for a group of 65 male rats selected for high efficiency of food utilization than for another group of 146 males fed the same diet under

similar environmental conditions but unselected insofar as their efficiency of food utilization was concerned.

The significant differences found between males and females in the percentages of the body dry matter, nitrogen, ash, and fat undoubtedly are important factors contributing to the marked differences in efficiency of food utilization between the sexes. The work of Slonaker (1912) has been cited by Mitchell and Carmen (1926) as evidence in support of the idea that the female rat is more active than the male; they conclude, therefore, that this greater activity may result in a lower efficiency for the former. The data recorded by Slonaker (1912) give results on only four rats, three males and one female. After the age of 3 months the one female showed much more activity than the males; for ages of less than 3 months, however, there was very slight differences in the activity of the two sexes. His data can not be used to explain, even in part, the lower efficiency of the female rats in the present investigation for the rats studied were all less than 2½ months old at the termination of the experimental period. Hitchcock (1927) studied the relationship of the total voluntary activity of rats, as measured by the revolving cages described by Hoskins (1925) and Durant (1924, 1925) (cited by Hitchcock, 1927) and the food consumption measured in calories. He did not distinguish between the sexes, but he observed a high degree of individual variation from which he concluded that there were factors other than the animal's activity which determined food consumption. Further studies are now in progress which it is believed will give some very interesting information on the activity of male and female rats in relation to the differences they show in their food utilization.

A study of Figure 13 and the data in Table 10 show that, when the efficiency quotient for the female is calculated on the body weight basis, the average efficiency index secured at the end of the first week is as reliable as one secured over a longer period. In fact, the variability of the female index is less the first week than for any of the succeeding periods studied. It is probable that this increased variability is to some extent related to the sexual development of the female. On the other hand, the male efficiency index, Table 9, decreases in variability for each week increase in the experimental period. The change after the third week, however, is not significant so it may be concluded that an efficiency index for a male rat may be satisfactorily determined during a period of three weeks. It seems that an application of the efficiency index in conjunction with food consumption as an aid in the interpretation of experimental data would necessitate the determination of the efficiency indices of the animals in a fore period of from one to

three weeks in length on the control diet. If possible, only animals should be selected that exhibit similar efficiency indices for this fore period. It is believed that such a procedure will insure more uniformity in the results obtained on diets differing by one variable and will aid materially in ascribing quantitative differences to such diets. Further inbreeding experiments are planned for the purpose of attempting to increase the uniformity of food utilization, but until such results are forthcoming it is believed advisable to test the efficiency of the animals by a fore period.

SUMMARY

A cross was made between a pair of unrelated rats which had widely different levels of efficiency of food utilization as shown by their efficiency indices. In general, the efficiency indices of the progeny appeared intermediate to that of their parents. The F_2 generation produced by various combinations of matings of the F_1 showed a much greater range in the variability than was found in the P_1 animals. Pairs of rats from the F_2 generation were selected and mated according to their high or low efficiency indices. The present report concerns two lines carried to the F_9 generation, one line descendant from a pair selected for high efficiency and the other from a pair selected for low efficiency. Sib matings were followed throughout. The variability in the efficiency indices of the supposedly different lines was studied and compared with the variability of the F_2 individuals.

All of the rats, between 500 and 600 individuals, were fed on the same diet, one which is believed to provide for a maximum rate of growth. The diet was fed individually and an accurate record was kept of the food consumption and gain in weight.

Animals with different efficiency indices were killed with chloroform and the length of their intestines was measured. In some cases after the intestinal tract had been emptied of its contents, the empty carcass was frozen, minced, and analyzed for moisture, fat, nitrogen, ash, and fat-free dry matter.

Significant differences were obtained for the efficiency indices of the two sexes; therefore a factor was determined that would convert the male efficiency indices to a scale comparable to that of the female.

A larger average gain in live weight on a lower average food consumption accompanied by less variability in both gain and food consumed was obtained for a group of 65 male rats in a line selected for high efficiency of food utilization than was obtained for a group of 146 male rats composed largely of an F_2 population in which selection had not been practiced.

Two lines of rats selected for different levels of efficiency of food utilization were produced. Significant differences in food utilization of these two lines were shown by means of the efficiency index method. The lower efficiency line is about 40% less efficient than the high efficiency line and is more variable.

Various statistical studies were made from the data obtained on the live animals, their food consumption, and measurements of their carcasses. The significance of the relationships discovered is pointed out. Methods are suggested for securing greater uniformity particularly in laboratory animals used for growth studies, and tests are now under way of the applicability of the plans outlined.

CONCLUSIONS

1. Evidence is given to support the belief that heritable factors influence the efficiency of food utilization. The nature of these factors is complex, insofar as they can be ascertained from this study.

2. There is a decided difference between the two sexes in the efficiency with which they utilize the food of an identical dietary.

3. The female rat consumes much more dry matter per unit of gain per unit of body weight than does the male when the measurements are made during the period in which the animals are making the most rapid growth—the average female efficiency index being 1.7 times that of the male during a 6-weeks period.

4. The chemical analyses of the carcasses show the female rat to be higher in dry matter, ash, and fat, and lower in nitrogen and fat-free dry matter than the male. This difference may account in part for the lower efficiency index of the female.

5. With the rat the correlation coefficient between dry matter consumed and gain in live weight for a 6-weeks experimental period for either sex is not high enough to warrant the use of the "paired-feeding" trial method for evaluating quantitatively the growth-promoting qualities of dietaries differing in only one constituent when a small number of animals are used.

6. There appears to be no statistically significant relationship between the length of the intestines and the amount of dry matter consumed by rapidly growing rats.

7. The efficiency index calculated from the grams of dry matter consumed per gram of gain in live weight per unit of surface area follows more closely the weekly increasing values of the ratio of food over gain than does the efficiency index calculated per unit of body weight. This probably means that the maintenance cost is the largest item involved in the efficiency of food utilization.

8. A higher correlation coefficient appears to exist for males than for females between the efficiency of food utilization of the first 3 weeks and the last 3 weeks of a 6-weeks experimental period.

9. Male rats show less variability in the efficiency indices in two 3-weeks periods than do females for similar periods.

10. Because of the two preceding conclusions it is believed that the male rat is more desirable than the female for use in growth studies.

11. Because a selected group of male rats was less variable than an unselected group in both food consumption and gain in live weight, it is believed that greater uniformity in these factors may be secured in these laboratory animals through inbreeding and selection.

12. The average level of efficiency of food utilization in the rats studied appears fairly uniform after the F_6 generation in both efficiency lines, altho there is some indication of a gradual decline in average efficiency in the lower efficiency line.

LITERATURE CITED

Beard, Howard H.
1926 Studies in the Nutrition of the White Mouse. I. The Normal Growth and Nutritive Requirements. Am. J. Physiol. **75**:645-657.

Benedict, F. G. and MacLeod, Grace
1929 The Heat Production of the Albino Rat. II. Influence of Environmental Temperature, Age, and Sex; Comparison with the Basal Metabolism of Man. J. Nut. **1**:367-398.

Blunt, Katharine and Cowan, Ruth
1930 Ultra Violet Light and Vitamin D in Nutrition. University of Chicago Press, pp. 2, 3.

Cowgill, George R.
1923 An Improved Procedure for Metabolism Experiments. J. Biol. Chem. **57**:725-737.

1928 The Energy Factor in Relation to Food Intake Experiments on the Dog. Am. J. Physiol. **85**:45-64.

Crampton, E. W.
1928 Individual Feeding for Comparative Feeding Trials with Hogs. MacDonald College, McGill Univ., Tech. Bull 5.

1931 Statistical Analysis of Comparative Feeding Trial Data. Scientific Agr. **11**:281-285.

Dawbarn, M. C.
1928 The Growth and Maintenance of White Mice Fed upon Synthetic Diets. Aust. Jour. Exp. Biol. and Med. Sc. **5**:149-169.

Evvard, J. M., Nelson, V. E., and Sewell, W. E.
1928 Copper Salts in Nutrition. Proc. Iowa Acad. Sc. **35**:211-215.

Goto, K.
1923 Beitrag zur Kenntnis der chemischen Wärmeregulation die Säugetiere. III. Wärmeregulation der weissen Ratte. Biochhem. Zeitschr. **135:** 107-121.

Griffith, Wendell H.
1929 The Effect of Liver and of Yeast on the Consumption and the Utilization of Food. Abs. of Communications, 13th International Physiol. Congress. Boston, p. 105.

———
1929a Nutritional Factors Affecting Growth of Rats on Diets Containing Sodium Benzoate. Proc. Soc. Exp. Biol. and Med. **26:**858-860.

———
1929b Benzoylated Amino Acids in the Animal Organism. IV. A Method for the Investigation of the Origin of Glycine. J. Biol. Chem. **82:** 415-427.

———
1930 Benzoylated Amino Acids in the Animal Organism. V. The Synthesis of Glycine and of Hippuric Acid in Rats. J. Biol. Chem. **85:** 751-758.

Harris, J. A.
1909 The Correlation Between a Variable and the Deviation of a Dependent Variable from Its Probable Value. Biometrika, **6:**438-441.

Hitchock, F. A.
1927 The Total Energy Requirement of the Albino Rat for Growth and Activity. Am. Jour. Physiol. **83:**28-36.

Hopkins, F. G.
1912 Feeding Experiments Illustrating the Importance of Accessory Factors in Normal Dietaries. J. Physiol. **44:**425-460.

Irwin, Margaret H.
1932 Studies in Vitamin A. Technic. Iowa State College. Jour. of Science **6:**451-454.

Lee, M. O. and Clark, Elizabeth
1929 Determination of the Surface Area of the White Rat with Its Application to the Expression of Metabolic Results. Am. J. Physiol. **89:**24-33.

Levine, H. and Smith, A. H.
1927 Growth Experiments on Diets Rich in Fats. J. Biol. Chem. **72:**223-238.

Macallum, A. B.
1919 The Relation of Vitamines to the Growth of Young Animals. Trans. Royal Canad. Inst. Toronto, **11:**175-241.

Mitchell, H. H.
1930 The Paired Feeding Method: Its Value and Limitations in Livestock Experimentation. Proc. Soc. Animal Prod. pp. 56-63.

Mitchell, H. H., and Beadles, J. R.
1930 The Paired Feed Method in Nutrition Experiments and Its Application to the Problem of Cystine Deficiencies in Food Proteins. Jour. Nut. **2:**225-244.

Mitchell, H. H., Card, L. E., and Hamilton, T. S.
1926 The Growth of White Plymouth Rock Chickens. Ill. Agr. Expt. Sta. Bull. 278.

Mitchell, H. H., and Carmen, G. G.
1926a The Composition of the Gains in Weight and the Utilization of Food Energy in Growing Rats. Am. Jour. Physiol. 76:398-410.
Mitchell, H. H., and Hamilton, T. S.
1927-28 Steer and Heifer Carcasses Analyzed Chemically. Ill. Agr. Expt. Sta. 41st Annual Rept. 99-100.
Morgan. T. H.
1926 Genetics and the Physiology of Development. Am. Nat. 60:489-515.
Osborne, T. B. and Mendel, L. B.
1926 The Relation of the Rate of Growth to Diet. I. Jour. Biol. Chem. 69:661-673.
Palmer, Leroy S., and Kennedy, Cornelia
1927 The Fundamental Food Requirements for the Growth of the Rat. I. Growth on a Simple Diet of Purified Nutrients. Jour. Biol. Chem. 74:591-611.

———

1929 The Fundamental Food Requirements for the Growth of the Rat. V. Influence of Fat in the Diet. Proc. Soc. Exp. Biol. and Med. 26:427-430.

———

1931 The Fundamental Food Requirements for the Growth of the Rat. VI. Influence of the Food Consumption and the Efficiency Quotient of the Animal. Jour. Biol. Chem. 90:545-564.
Slonaker, J. R.
1912 The Normal Activity of the Albino Rat from Birth to Natural Death, Its Rate of Growth and the Duration of Life. Jour. Animal Behavior, 2:20-42.
Smith, A. H. and Carey, Elizabeth
1923 Growth on Diets High in Carbohydrate and High in Fat. Jour. Biol. Chem. 58:425-433.
"Student"
1908 The Probable Error of the Mean.
1917 Biometrika 6:1-25.
Biometrika 11:414-417.
Thompson, Helen B.
1926 The Effect of Low Ash Feeding in the Growth of the Albino Mouse, with Special Reference to the Economy of Food Consumption. Am. J. Physiol. 77:140-157.
Titus, H. W., McNally, Edmund, and Hilberg, F. C.
1930 A Comparison of Shrimp "Bran" and Two Kinds of Fish Meal when Fed at a Level of Ten per Cent in Diets for Growing Chicks. Poult. Sc. 9:219-234.
Wright, S.
1921 Systems of Mating. Jour. Genetics, 6:111-178.